

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

GOLDING, Louise
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
ROYAUME-UNI

Date of mailing (day/month/year) 20 April 2001 (20.04.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 11.69118/002	
International application No. PCT/GB99/03488	International filing date (day/month/year) 22 October 1999 (22.10.99)

1. The following indications appeared on record concerning:		
<input checked="" type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input type="checkbox"/> the agent <input type="checkbox"/> the common representative
Name and Address GOLDING, Louise 179 Queen Victoria Street London EC4V 4EL United Kingdom	State of Nationality GB	State of Residence GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input checked="" type="checkbox"/> the person	<input type="checkbox"/> the name	<input type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence
Name and Address NYCOMED IMAGING AS Nycoveien 1-2 N-0401 Oslo Norway	State of Nationality NO	State of Residence NO
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary: The person appearing in Box 1 above has assigned all rights in connection with the above application to the person appearing in Box 2.		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer R. Chrem
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 June 2000 (20.06.00)	
International application No. PCT/GB99/03488	Applicant's or agent's file reference 11.69118/002
International filing date (day/month/year) 22 October 1999 (22.10.99)	Priority date (day/month/year) 22 October 1998 (22.10.98)
Applicant KELLAR, Kenneth	

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

22 May 2000 (22.05.00)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

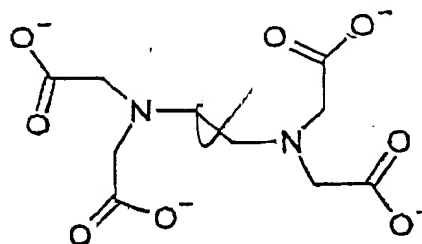
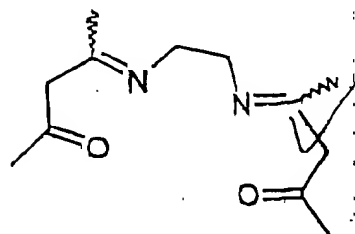
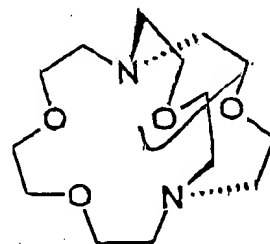
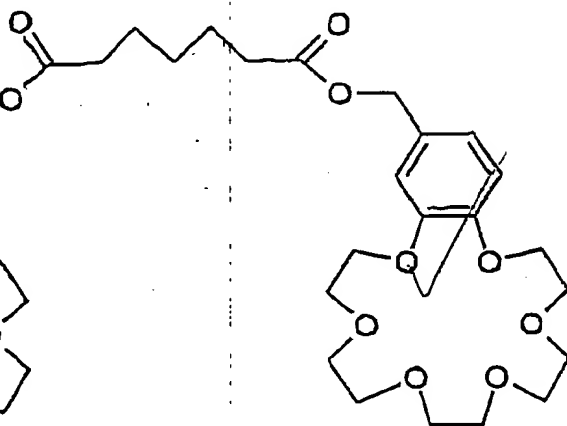
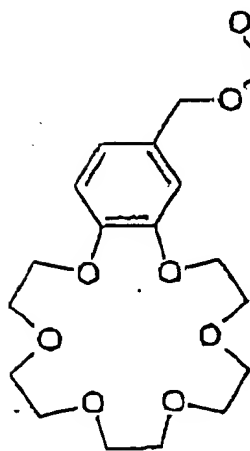
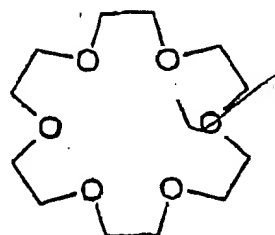
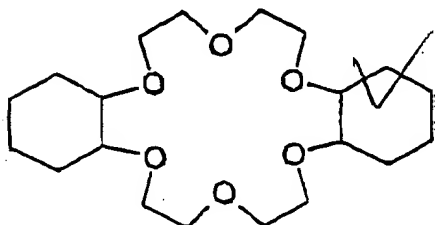
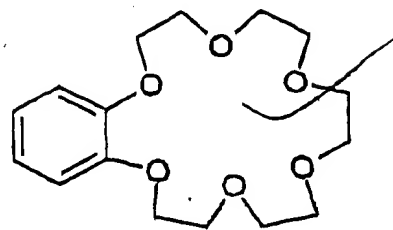
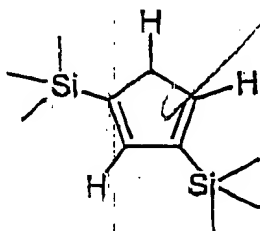
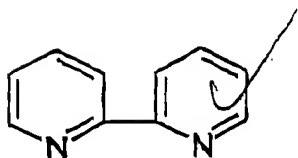
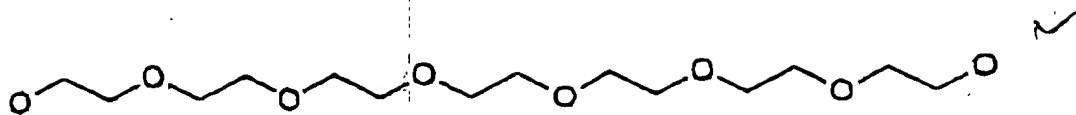
<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No.: (41-22) 740.14.35</p>	<p>Authorized officer</p> <p>Olivia RANAIVOJAONA</p> <p>Telephone No.: (41-22) 338.83.38</p>
--	--

CompoundREPLACED BY
ART 34 AMDT

5 This invention relates to compounds useful as contrast agents in magnetic resonance imaging and to methods of imaging using such compounds.

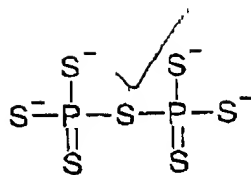
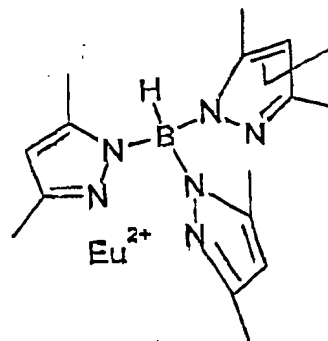
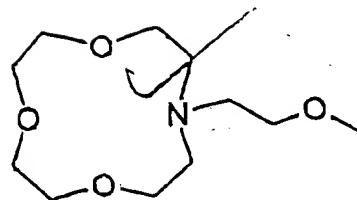
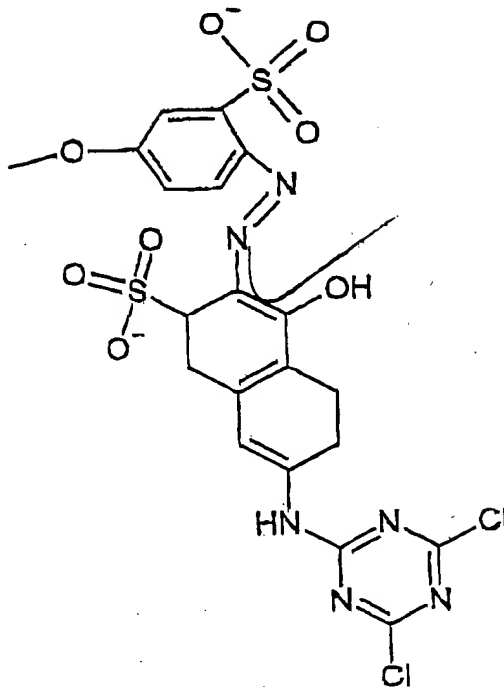
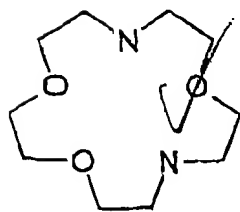
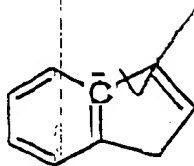
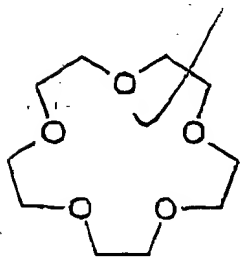
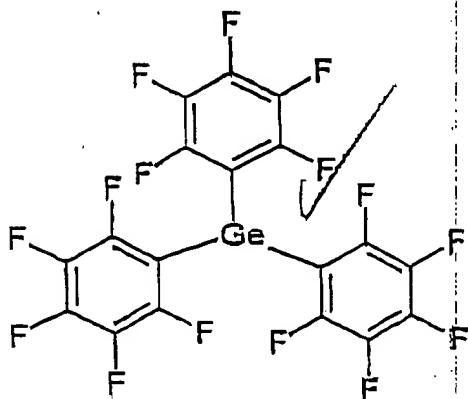
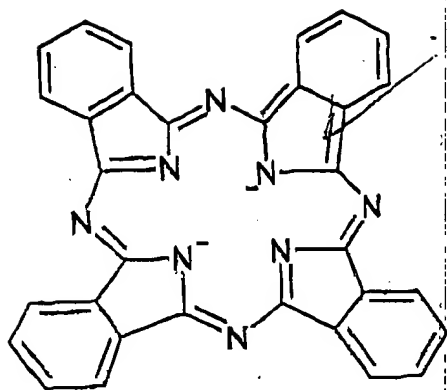
10 Magnetic resonance (MR) imaging is a well established imaging modality in which the image is derived from the intensity of the nmr signal from protons (usually water protons) in the subject under study. Because most tissue has an approximately 80% water content, contrast in MR imaging is attained by the application of pulse sequences that reveal differences in the relaxation times (T_1 and T_2) of the tissues. As
15 with other diagnostic imaging modalities such as CT and ultrasound, contrast agents may be used in MR imaging procedures to enhance contrast in the images produced, e.g. to allow clearer differentiation between different tissue types or between healthy and non-healthy tissue.
20 In MR imaging, the contrast agents conventionally are chelated paramagnetic species (e.g. Gd DTPA, Gd DTPA-BMA and Gd HP-DO3A, available commercially under the trade names Magnevist, Omniscan and Pro-Hance), which achieve contrast enhancement because of their relaxivities, their ability to decrease the relaxation times of water
25 protons.

A proposal has been made, in WO96/38184, that "triggered" paramagnetic metal ion complexes be used as MR contrast agents. As described in WO96/38184, the
30 trigger mechanism has the paramagnetic complex being "turned on" as an MR contrast agent by the presence of a target substance which interacts with the agent complexing the paramagnetic metal ion so as to free an inner sphere coordination site and allow water molecule exchange to take place at the freed-up site. In the
35 absence of the target substance, the complexed paramagnetic metal ion has no inner sphere coordination



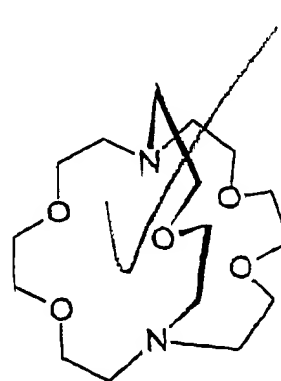
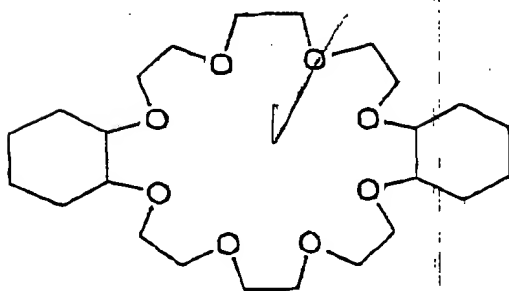
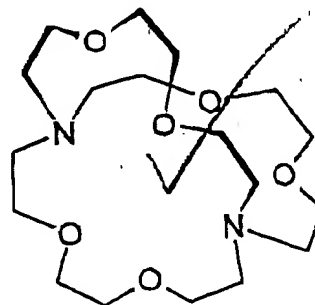
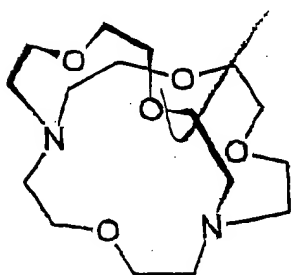
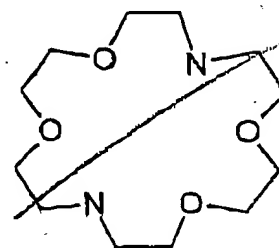
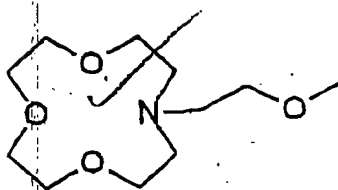
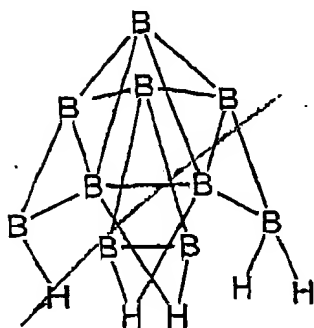
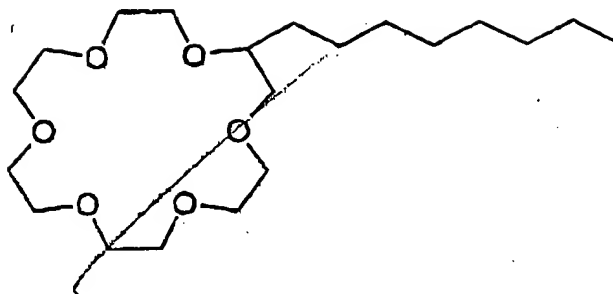
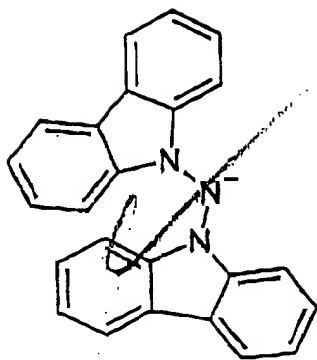
SUBSTITUTE SHEET (RULE 26)

oh

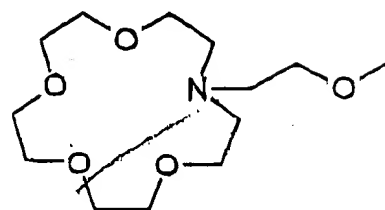
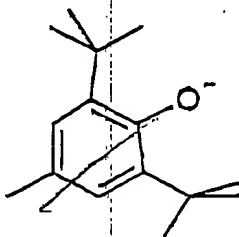
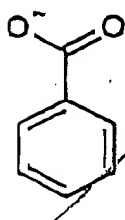
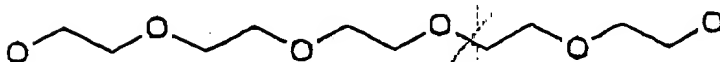
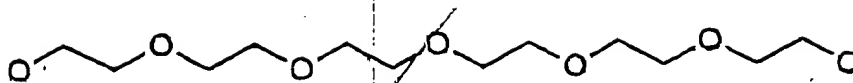


SUBSTITUTE SHEET (RULE 26)

or



ch



Ch

SUBSTITUTE SHEET (RULE 26)

Certain complexing agents may affect the redox couple and may stabilise the metal in a higher or lower oxidation state. The complexing agent may also significantly affect the biodistribution. For example
5 depending on the charge on the metal ion and the degree of ionisation of the complexing agent, the metal complexes of use in the present invention may be charged or neutral. Neutral complexes, which do not carry highly hydrophilic substituents may be sufficiently
10 lipophilic to cross lipid membranes such as cell membranes or the blood-brain barrier. Lipophilicity can be readily adjusted by varying the nature of the complexing agent and will be readily achieved by the skilled person. The change in relaxivity in switching
15 between different oxidation states may be further enhanced by having as the first oxidation state a very high relaxivity compound, such as a polymeric chelate of the lanthanide metal ion, or a rigid paramagnetic polychelate of the lanthanide metal ion, e.g. a vector
20 targeted lanthanide chelate or polychelate, or a dendrimeric chelate of a lanthanide metal ion such as described in WO93/06868 with a short linkage or a hydrophobic linkage between dendrimeric branching sites. Further enhancement of the sensitivity of the "on-off"
25 switch may be achieved by having as the second oxidation state a very low relaxivity compound. This may be achieved, for example, by having as the low relaxivity compound the same material with the lanthanide metal present in the second oxidation state and (i) with water
30 coordination sites reversibly blocked by an enzymically removable blocking group (e.g. as described in WO96/38184) and/or (ii) with a targeting vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif such as RGD) which in that state is not bound to
35 its intended substrate.

Thus the switching between low and high relaxivity states may be further enhanced by binding of the

targeting vector or displacement of the blocking groups or alternatively by interaction at the target site to change a high relaxivity conformation into a low relaxivity conformation, or vice versa. Such conformational changes can be achieved for the compounds of PCT/GB96/01308 by changing the chemical nature of their immediate environment (e.g. by the presence or addition of urea).

Preferably, the contrast agent for use in accordance with the invention may be further conjugated to a macromolecule. In this way the relaxivity of the contrast agent is further increased thereby enhancing the sensitivity of the "on-off" switch between relaxivity states. Examples of suitable macromolecules include proteins and polymers, e.g. that prepared in accordance with Example 2 of WO98/10797, and macrostructures such as liposomes in which the chelate is bound to the outer surface.

The means by which conversion from one oxidation state to another may be achieved may be a biological process or malfunction. Accordingly, the method of the invention finds application in methods of "functional" MR imaging capable of providing vital information relating to the functioning of particular parts of the body. For example, the method of the invention can be used to identify parts of the body which may be functioning abnormally, e.g. as a result of disease.

Transition between the "on" and "off" states may, for example, result from the presence or absence of oxygen or of oxidation or reduction promoting agents, from a change in temperature or as a result of an increase or decrease in pH at the target site, or as a result of the presence of a specific enzyme. For example, in the case of an "on-off" system, the MR image will appear bright unless there is a specific condition present such as oxygen deficiency which causes switching of the contrast agent to the lower relaxivity state and

a corresponding reduction of image intensity to that which would be expected in the absence of the contrast agent.

Alternatively the means for conversion may be a chemical agent administered to the subject, e.g. a redox reagent capable of delivery to or accumulation at a desired target site within the body, or designed for release at such a site for example a tumour or oedema. In some cases, activation of the agent may involve application of light, preferably with a wavelength of from 600 to 1300 nm in order to minimise absorption by the body.

As mentioned above, in one aspect of the invention the relaxivity of the contrast agent may be switched as a result of a change in pH. Contrast agents for use in the method of the invention may thus be used to detect areas of the body which are acidic or basic due to physiological or disease processes. Typically, they may be used to detect regions of pH of about 4 to ~5.5 within the body by appropriate selection as the contrast agent of a substance having a pKa value above or below a predictable threshold.

For example, many tumors exhibit a lower extracellular pH, e.g. as low as 5.5, typically between ~5.5 and ~7.7. This is a result of decreased vascular perfusion resulting in chronic hypoxia and increased lactic acid levels, exacerbated by the typically higher metabolic rate of cancerous cells.

Certain metal complexes undergo more rapid hydrolysis at lower pH, e.g. those of formula (VI) above as described in W098/39288 which is herein incorporated by reference. Due to the rapid hydrolysis, metal ions are selectively trapped in the area of low pH allowing targeting of the metal ion to certain areas of the body.

On the other hand, necrotic areas within tumors may exhibit a higher, more basic, pH. Acidic tumor types which may be detected using the method of the invention

include malignant melanoma, squamous cell carcinoma, sarcomas and adenocarcinomas (see Thistlewaite et al., Int. J. Radiation Oncology Biol. Phys. 11: 1647-1652, 1985).

5 Osteoporosis is a degenerative bone disorder. During the physiological process of bone resorption osteoclasts excavate small pits throughout the bone, creating a zone of reduced pH between the osteoclast and the bone tissue. pH values as low as 4.0 have been
10 measured in the active erosion zones (see Silver et al., Cell Res. 175: 266-267, 1988). Effective imaging of this erosion zone using the method of the invention may be used to provide vital information regarding the effectiveness of therapies used in the treatment of
15 osteoporosis. In this way, the clinician may readily determine the therapeutic effect of a given drug and use the information either to continue therapy or to change therapies.

Measurement of local osteoclastic activity using
20 the method of the invention may also be used to evaluate other bone remodelling activities such as the repair of fractures, the treatment of Paget's disease or to evaluate the extent of expanding lesions in bone, such as tumors, in which resorption may take place at the
25 bone surface in contact with the lesion.

In the method of the invention, the region in which the conversion from first to second relaxivity state occurs will preferably be identified, e.g. by comparison with a "native" image in the collection of which the
30 means for conversion has not been administered or activated or with a comparison body site in which the biological process responsible for the conversion does not occur.

The contrast agents for use in the method of the
35 invention, in particular those comprising chelating agents, may if desired be conjugated to biological vectors so as to target actively or passively to the

desired regions of the body. Conjugation of metal chelates to targeting vectors is discussed for example in WO93/21957 and US-A-5595725 (Schering).

5 Where the targeting vector is such as to bind the agent to a target site, relaxivity will be increased as a result and in one embodiment of the invention the triggering of enhanced relaxivity may be achieved by a combination of the freeing up of a coordination site according to WO96/38184 and binding to a larger
10 structure, e.g. a cell wall or the wall of a body duct using a vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif (such as RGD) conjugated to a compound according to WO96/38184.

15 The contrast agent for use in the method of the invention may be for example a complex of a lanthanide metal ion having first and second oxidation states and, in the high relaxivity state, at least one open coordination site for the exchange of water molecules. Such agents are capable of switching between first and
20 second relaxivity states as a result of a change in pH. In the case of Eu(II), a change in pH may be sufficient to alter the chelate so that this becomes very sensitive to oxygen concentration and so able to make the transition to Eu(III).

25 For administration into the GI tract, it may be unnecessary to chelate the metal and thus for this route simple salts, e.g. chlorides, may be used.

30 The contrast agent may be administered by any convenient route, eg. topical, transdermal, nasal, sublingual, oral, rectal, by direct instillation into an externally voiding body cavity (eg. lungs, uterus, GI tract and bladder), or subcutaneously, intramuscularly, interstitially or into the vasculature, eg. by injection or infusion. In general administration into the
35 vasculature or into the GI tract will be preferred routes.

For administration, the contrast agent may be

formulated together with appropriate conventional pharmaceutically acceptable carrier or excipients, such as liquid carriers (eg. saline or water for injections), pH and osmolality regulators, stabilizers, viscosity
5 modifiers, surfactants, bulking agents, skin penetration agents, flavourings, solid or semi-solid carriers (eg. hydrophilic gels), aerosol dispersants, etc.

The dose of contrast agent required will depend on the species and condition under study, the selected
10 contrast agent, and the administration route. However in general doses for i.v. administration will normally be in the range 0.001 to 5.0 mmol paramagnetic centre/kg bodyweight (where by paramagnetic centre is meant a metal atom which is or becomes paramagnetic).

15 If a chemical agent (a "trigger", for example an enzyme, a redox agent or a free radical scavenger) is administered to trigger the conversion between states of different relaxivity, then this can be administered together with the contrast agent or separately, eg.
20 before or after or even simultaneously in the event that the administration site is different. If coadministered, then the trigger may be formulated to contact the contrast agent only after delivery or on reaching the target site. Thus for example it may be
25 encapsulated in a matrix or membrane (e.g. a vesicle membrane) which breaks down or is broken down at the desired target site. If the method of the invention is used intraoperatively, e.g. to highlight damage to a particular tissue or organ or to delineate a mass to be
30 removed, the trigger may be applied to the operating site during the operation so as to switch on the contrast agent at the cutting site. In this event the trigger may be a chemical agent as discussed above, the air, or an applied physical stimulus.

35 The compositions containing both the contrast agent and a chemical trigger are themselves new and form a further aspect of the invention. Viewed from this

aspect the invention provides an MR contrast agent composition comprising as an MR contrast agent a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, preferably at least 10, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero, and which is convertible in vivo from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, together with an optionally encapsulated physiologically tolerable trigger substance capable of converting said contrast agent between said first and second oxidation states.

Viewed from a further aspect the invention also provides the use of a physiologically tolerable MR contrast agent substance comprising a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to the method of the invention.

All documents referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the accompanying non-limiting Examples.

Example 1

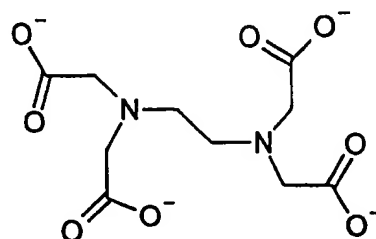
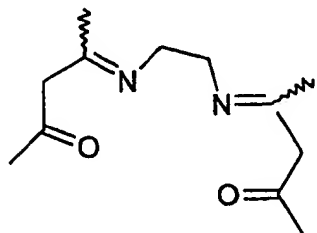
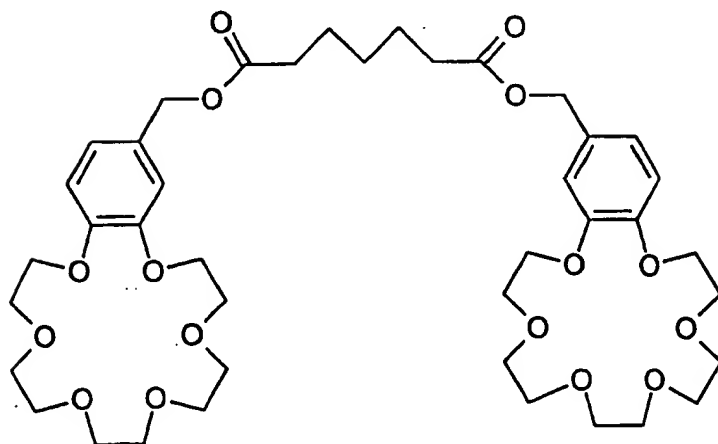
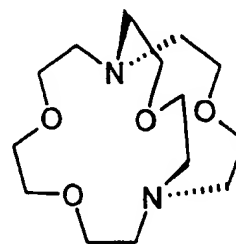
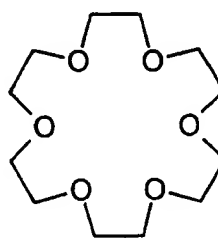
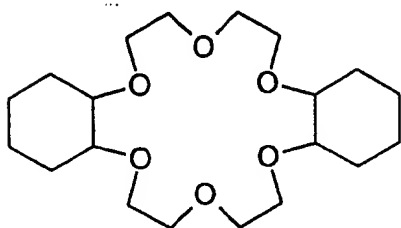
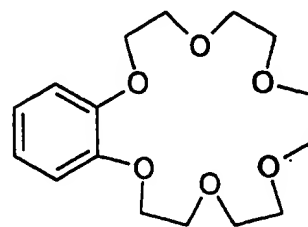
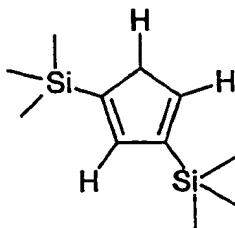
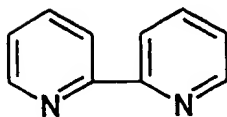
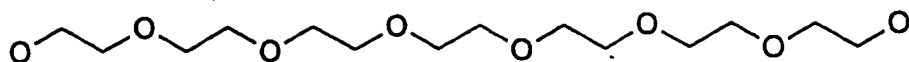
This example involves the use of a Eu(II)-chelate for detecting a low oxygen concentration in a tumor. An Eu(II)-chelate would be injected intravenously and a T₁-

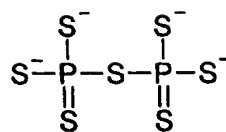
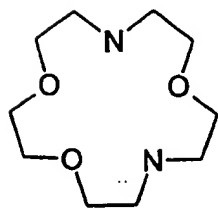
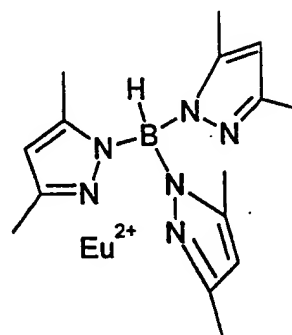
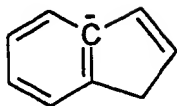
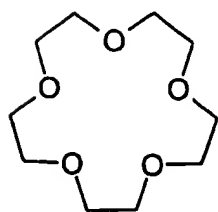
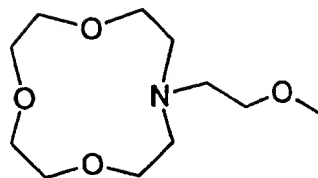
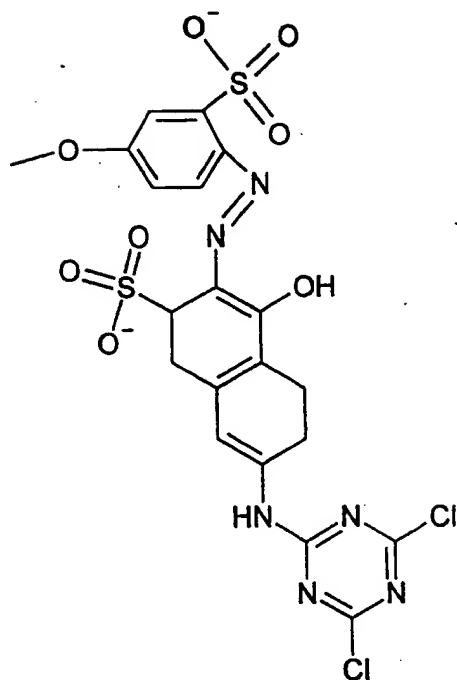
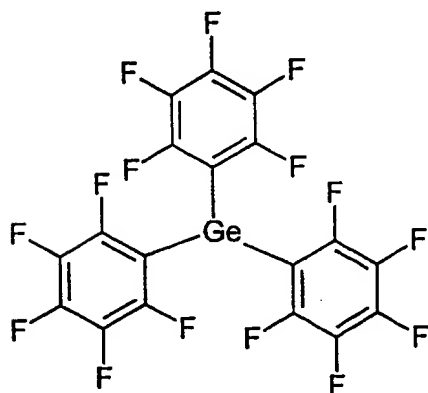
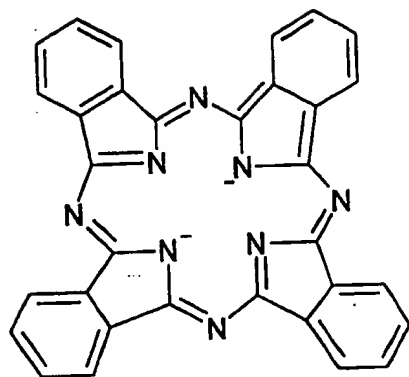
weighted pulse sequence would be used to obtain the images for a variety of times post injection of the Eu(II)-chelate. In the first image obtained at a time immediately after injection, the blood pool containing the Eu(II)-chelate would appear as being bright (high signal intensity). As time elapses, the Eu(II)-chelate will distribute into tissue, and the Eu(II) will oxidise to Eu(III) at a rate that depends on the local oxygen concentration. As a result of the oxidation, the signal intensity of images obtained at later times of regions containing the Eu-chelate will decrease as more Eu(III) is formed. The signal intensity will decrease more rapidly with time in regions of high oxygen concentration. Therefore, regions of low oxygen concentration will have a signal intensity that decreases more slowly with time, and these regions could eventually appear as bright spots on the images. Such a sensitivity to oxygen concentration could prove very useful in the characterisation of tumors, for example. The sensitivity to oxygen concentration could also prove useful in the evaluation of cardiac tissue and possibly stroke as well. However, in the evaluation of stroke and/or brain perfusion, it may be useful to use a T_2 -weighted pulse sequence.

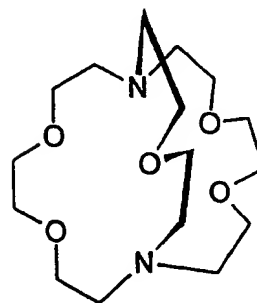
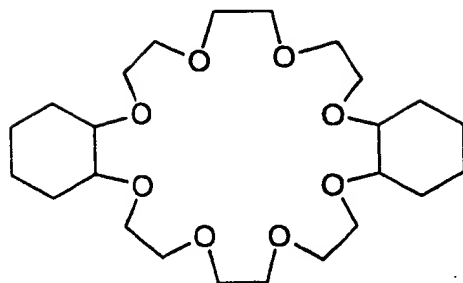
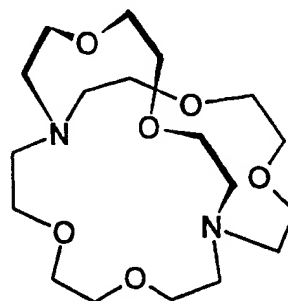
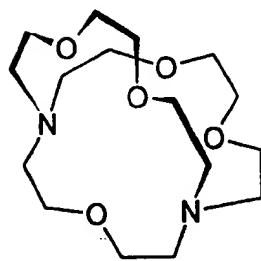
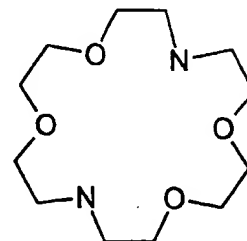
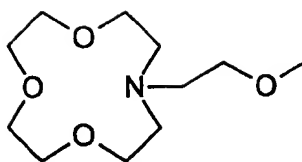
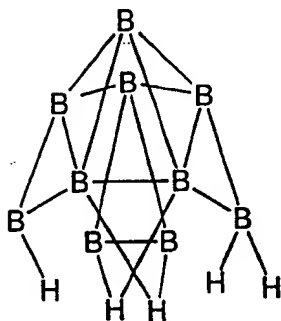
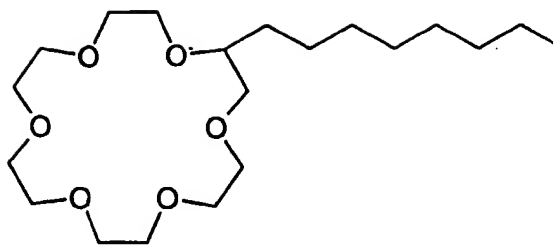
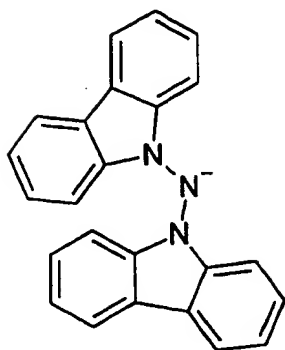
Example 2

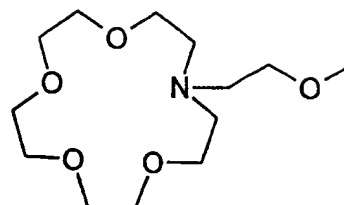
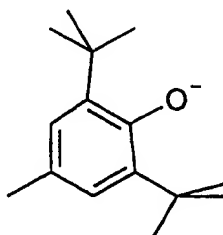
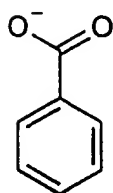
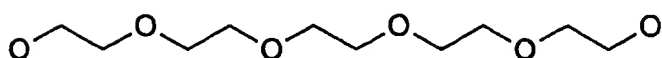
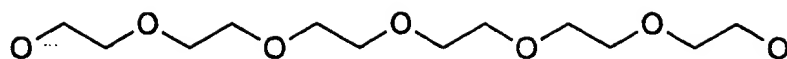
This example involves the use of an Eu(II)-chelate conjugated to a macromolecule, designed for characterising the oxygen content of a tumor. As an example, the Eu(II)-chelate-macromolecule complexes are similar to the Gd(III)-chelate-macromolecule complexes described in T.S. Desser, K.I. Rubin, H.H. Muller, F. Qing, S. Khodar, G. Zanazzi, S.W. Young, D.L. Ladd, J.A. Wellons, K.E. Kellar, J.L. Toner, R.A. Snow, "Dynamics of Tumor Imaging with Gd-DTPa-Polyethylene Glycol Polymers: Dependence on Molecular Weight" Journal of

Magnetic Resonance Imaging 4, 467-472 (1994), where
Eu(II) takes the place of Gd(III). In this work,
macromolecular complexes have been shown to have an
extended lifetime in the blood pool as a result of
5 conjugating the metal chelate to a polymer, and this
increased lifetime enables the complexes to be taken up
by tumors. However, unlike their Gd(III)-containing
counterparts, the Eu(II)-containing macromolecular
complexes will be sensitive to the oxygen content of the
10 tumors as described in Example 1 above.









Certain complexing agents may affect the redox couple and may stabilise the metal in a higher or lower oxidation state. The complexing agent may also significantly affect the biodistribution. For example
5 depending on the charge on the metal ion and the degree of ionisation of the complexing agent, the metal complexes of use in the present invention may be charged or neutral. Neutral complexes, which do not carry highly hydrophilic substituents may be sufficiently
10 lipophilic to cross lipid membranes such as cell membranes or the blood-brain barrier. Lipophilicity can be readily adjusted by varying the nature of the complexing agent and will be readily achieved by the skilled person. The change in relaxivity in switching
15 between different oxidation states may be further enhanced by having as the first oxidation state a very high relaxivity compound, such as a polymeric chelate of the lanthanide metal ion, or a rigid paramagnetic polychelate of the lanthanide metal ion, e.g. a vector
20 targeted lanthanide chelate or polychelate, or a dendrimeric chelate of a lanthanide metal ion such as described in WO93/06868 with a short linkage or a hydrophobic linkage between dendrimeric branching sites. Further enhancement of the sensitivity of the "on-off"
25 switch may be achieved by having as the second oxidation state a very low relaxivity compound. This may be achieved, for example, by having as the low relaxivity compound the same material with the lanthanide metal present in the second oxidation state and (i) with water
30 coordination sites reversibly blocked by an enzymically removable blocking group (e.g. as described in WO96/38184) and/or (ii) with a targeting vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif such as RGD) which in that state is not bound to
35 its intended substrate.

Thus the switching between low and high relaxivity states may be further enhanced by binding of the

targeting vector or displacement of the blocking groups or alternatively by interaction at the target site to change a high relaxivity conformation into a low relaxivity conformation, or vice versa. Such

5 conformational changes can be achieved for the compounds of PCT/GB96/01308 by changing the chemical nature of their immediate environment (e.g. by the presence or addition of urea).

Preferably, the contrast agent for use in

10 accordance with the invention may be further conjugated to a macromolecule. In this way the relaxivity of the contrast agent is further increased thereby enhancing the sensitivity of the "on-off" switch between relaxivity states. Examples of suitable macromolecules

15 include proteins and polymers, e.g. that prepared in accordance with Example 2 of WO98/10797, and macrostructures such as liposomes in which the chelate is bound to the outer surface.

The means by which conversion from one oxidation

20 state to another may be achieved may be a biological process or malfunction. Accordingly, the method of the invention finds application in methods of "functional" MR imaging capable of providing vital information relating to the functioning of particular parts of the

25 body. For example, the method of the invention can be used to identify parts of the body which may be functioning abnormally, e.g. as a result of disease.

Transition between the "on" and "off" states may, for example, result from the presence or absence of

30 oxygen or of oxidation or reduction promoting agents, from a change in temperature or as a result of an increase or decrease in pH at the target site, or as a result of the presence of a specific enzyme. For example, in the case of an "on-off" system, the MR image

35 will appear bright unless there is a specific condition present such as oxygen deficiency which causes switching of the contrast agent to the lower relaxivity state and

a corresponding reduction of image intensity to that which would be expected in the absence of the contrast agent.

Alternatively the means for conversion may be a chemical agent administered to the subject, e.g. a redox reagent capable of delivery to or accumulation at a desired target site within the body, or designed for release at such a site for example a tumour or oedema. In some cases, activation of the agent may involve application of light, preferably with a wavelength of from 600 to 1300 nm in order to minimise absorption by the body.

As mentioned above, in one aspect of the invention the relaxivity of the contrast agent may be switched as a result of a change in pH. Contrast agents for use in the method of the invention may thus be used to detect areas of the body which are acidic or basic due to physiological or disease processes. Typically, they may be used to detect regions of pH of about 4 to ~5.5 within the body by appropriate selection as the contrast agent of a substance having a pKa value above or below a predictable threshold.

For example, many tumors exhibit a lower extracellular pH, e.g. as low as 5.5, typically between ~5.5 and ~7.7. This is a result of decreased vascular perfusion resulting in chronic hypoxia and increased lactic acid levels, exacerbated by the typically higher metabolic rate of cancerous cells.

Certain metal complexes undergo more rapid hydrolysis at lower pH, e.g. those of formula (VI) above as described in WO98/39288 which is herein incorporated by reference. Due to the rapid hydrolysis, metal ions are selectively trapped in the area of low pH allowing targeting of the metal ion to certain areas of the body.

On the other hand, necrotic areas within tumors may exhibit a higher, more basic, pH. Acidic tumor types which may be detected using the method of the invention

include malignant melanoma, squamous cell carcinoma, sarcomas and adenocarcinomas (see Thistlewaite et al., Int. J. Radiation Oncology Biol. Phys. 11: 1647-1652, 1985).

5 Osteoporosis is a degenerative bone disorder. During the physiological process of bone resorption osteoclasts excavate small pits throughout the bone, creating a zone of reduced pH between the osteoclast and the bone tissue. pH values as low as 4.0 have been
10 measured in the active erosion zones (see Silver et al., Cell Res. 175: 266-267, 1988). Effective imaging of this erosion zone using the method of the invention may be used to provide vital information regarding the effectiveness of therapies used in the treatment of
15 osteoporosis. In this way, the clinician may readily determine the therapeutic effect of a given drug and use the information either to continue therapy or to change therapies.

 Measurement of local osteoclastic activity using
20 the method of the invention may also be used to evaluate other bone remodelling activities such as the repair of fractures, the treatment of Paget's disease or to evaluate the extent of expanding lesions in bone, such as tumors, in which resorption may take place at the
25 bone surface in contact with the lesion.

 In the method of the invention, the region in which the conversion from first to second relaxivity state occurs will preferably be identified, e.g. by comparison with a "native" image in the collection of which the
30 means for conversion has not been administered or activated or with a comparison body site in which the biological process responsible for the conversion does not occur.

 The contrast agents for use in the method of the
35 invention, in particular those comprising chelating agents, may if desired be conjugated to biological vectors so as to target actively or passively to the

desired regions of the body. Conjugation of metal chelates to targeting vectors is discussed for example in WO93/21957 and US-A-5595725 (Schering).

5 Where the targeting vector is such as to bind the agent to a target site, relaxivity will be increased as a result and in one embodiment of the invention the triggering of enhanced relaxivity may be achieved by a combination of the freeing up of a coordination site according to WO96/38184 and binding to a larger
10 structure, e.g. a cell wall or the wall of a body duct using a vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif (such as RGD) conjugated to a compound according to WO96/38184.

The contrast agent for use in the method of the
15 invention may be for example a complex of a lanthanide metal ion having first and second oxidation states and, in the high relaxivity state, at least one open coordination site for the exchange of water molecules. Such agents are capable of switching between first and
20 second relaxivity states as a result of a change in pH. In the case of Eu(II), a change in pH may be sufficient to alter the chelate so that this becomes very sensitive to oxygen concentration and so able to make the transition to Eu(III).

25 For administration into the GI tract, it may be unnecessary to chelate the metal and thus for this route simple salts, e.g. chlorides, may be used.

The contrast agent may be administered by any convenient route, eg. topical, transdermal, nasal, sub-
30 lingual, oral, rectal, by direct instillation into an externally voiding body cavity (eg. lungs, uterus, GI tract and bladder), or subcutaneously, intramuscularly, interstitially or into the vasculature, eg. by injection or infusion. In general administration into the
35 vasculature or into the GI tract will be preferred routes.

For administration, the contrast agent may be

formulated together with appropriate conventional pharmaceutically acceptable carrier or excipients, such as liquid carriers (eg. saline or water for injections), pH and osmolality regulators, stabilizers, viscosity
5 modifiers, surfactants, bulking agents, skin penetration agents, flavourings, solid or semi-solid carriers (eg. hydrophilic gels), aerosol dispersants, etc.

The dose of contrast agent required will depend on the species and condition under study, the selected
10 contrast agent, and the administration route. However in general doses for i.v. administration will normally be in the range 0.001 to 5.0 mmol paramagnetic centre/kg bodyweight (where by paramagnetic centre is meant a metal atom which is or becomes paramagnetic).

15 If a chemical agent (a "trigger", for example an enzyme, a redox agent or a free radical scavenger) is administered to trigger the conversion between states of different relaxivity, then this can be administered together with the contrast agent or separately, eg.
20 before or after or even simultaneously in the event that the administration site is different. If coadministered, then the trigger may be formulated to contact the contrast agent only after delivery or on reaching the target site. Thus for example it may be
25 encapsulated in a matrix or membrane (e.g. a vesicle membrane) which breaks down or is broken down at the desired target site. If the method of the invention is used intraoperatively, e.g. to highlight damage to a particular tissue or organ or to delineate a mass to be
30 removed, the trigger may be applied to the operating site during the operation so as to switch on the contrast agent at the cutting site. In this event the trigger may be a chemical agent as discussed above, the air, or an applied physical stimulus.

35 The compositions containing both the contrast agent and a chemical trigger are themselves new and form a further aspect of the invention. Viewed from this

aspect the invention provides an MR contrast agent composition comprising as an MR contrast agent a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, preferably at least 10, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero, and which is convertible in vivo from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, together with an optionally encapsulated physiologically tolerable trigger substance capable of converting said contrast agent between said first and second oxidation states.

Viewed from a further aspect the invention also provides the use of a physiologically tolerable MR contrast agent substance comprising a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to the method of the invention.

All documents referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the accompanying non-limiting Examples.

Example 1

This example involves the use of a Eu(II)-chelate for detecting a low oxygen concentration in a tumor. An Eu(II)-chelate would be injected intravenously and a T₁-

weighted pulse sequence would be used to obtain the images for a variety of times post injection of the Eu(II)-chelate. In the first image obtained at a time immediately after injection, the blood pool containing the Eu(II)-chelate would appear as being bright (high signal intensity). As time elapses, the Eu(II)-chelate will distribute into tissue, and the Eu(II) will oxidise to Eu(III) at a rate that depends on the local oxygen concentration. As a result of the oxidation, the signal intensity of images obtained at later times of regions containing the Eu-chelate will decrease as more Eu(III) is formed. The signal intensity will decrease more rapidly with time in regions of high oxygen concentration. Therefore, regions of low oxygen concentration will have a signal intensity that decreases more slowly with time, and these regions could eventually appear as bright spots on the images. Such a sensitivity to oxygen concentration could prove very useful in the characterisation of tumors, for example. The sensitivity to oxygen concentration could also prove useful in the evaluation of cardiac tissue and possibly stroke as well. However, in the evaluation of stroke and/or brain perfusion, it may be useful to use a T₂-weighted pulse sequence.

Example 2

This example involves the use of an Eu(II)-chelate conjugated to a macromolecule, designed for characterising the oxygen content of a tumor. As an example, the Eu(II)-chelate-macromolecule complexes are similar to the Gd(III)-chelate-macromolecule complexes described in T.S. Dessler, K.I. Rubin, H.H. Muller, F. Qing, S. Khodar, G. Zanazzi, S.W. Young, D.L. Ladd, J.A. Wellons, K.E. Kellar, J.L. Toner, R.A. Snow, "Dynamics of Tumor Imaging with Gd-DTPa-Polyethylene Glycol Polymers: Dependence on Molecular Weight" Journal of

- 22 -

Magnetic Resonance Imaging 4, 467-472 (1994), where Eu(II) takes the place of Gd(III). In this work, macromolecular complexes have been shown to have an extended lifetime in the blood pool as a result of conjugating the metal chelate to a polymer, and this increased lifetime enables the complexes to be taken up by tumors. However, unlike their Gd(III)-containing counterparts, the Eu(II)-containing macromolecular complexes will be sensitive to the oxygen content of the tumors as described in Example 1 above.

Claims:

1. A method of generating a contrast enhanced image of a human or non-human animal subject which comprises
5 administering to said subject an effective amount of a magnetic resonance imaging contrast agent and generating an image of at least part of said subject containing said agent, wherein said agent comprises a physiologically tolerable lanthanide compound or salt
10 thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state
15 does or does not occur.
2. A method as claimed in claim 1 wherein said agent comprises a physiologically tolerable lanthanide compound or salt thereof having first and second
20 oxidation states which differ in relaxivity by a factor of at least 10.
3. A method as claimed in claim 1 wherein said agent comprises a physiologically tolerable lanthanide
25 compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 20.
4. A method as claimed in claim 1 wherein said agent
30 comprises a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 100.
- 35 5. A method as claimed in any one of claims 1 to 4 wherein the change between said first and said second oxidation states is effected as a change from a

paramagnetic to a diamagnetic state, as a change from a diamagnetic to a paramagnetic state, or as a change between two paramagnetic states of the lanthanide metal ion.

5

6. A method as claimed in claim 5 wherein said change between two paramagnetic states is effected as a change from a non-spherically symmetric electronic ground state to a spherically symmetric electronic ground state, or a
10 change from a non-spherically symmetric electronic ground state to a spherically symmetric excited state.

7. A method as claimed in any preceding claim wherein said agent is a chelate complex of a lanthanide metal
15 ion, or a physiologically tolerable salt thereof.

8. A method as claimed in any preceding claim wherein said agent is a Europium compound, preferably a chelate complex of Europium or a physiologically tolerable salt
20 thereof.

9. A method as claimed in claim 8 wherein said Europium compound is activated by switching between the II and III oxidation states of the metal ion.
25

10. A method as claimed in any one of claims 7 to 9 wherein said chelate complex is a complex of a chelant selected from the group consisting of DTPA, EDTA, DTPA-BMA, DO3A, DOTA, HP-DO3A, TMT and DPDP.
30

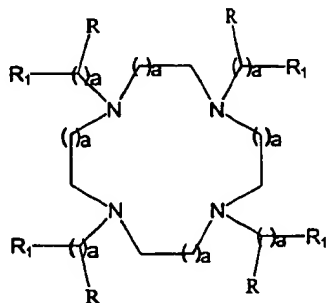
11. A method as claimed in any one of claims 7 to 9 wherein said chelate complex is a complex of a chelant selected from the group consisting of porphyrins and porphyrin-like molecules, phthalocyanines, crown ethers, hemin, heme, chelants having a square planar symmetry, cryptands and cryptates.
35

- 25 -

12. A method as claimed in any one of claims 7 to 9 wherein said chelate complex is a complex of a chelant selected from compounds of formulae (I), (II), (III), (IV), (V) and (VI):

5

10

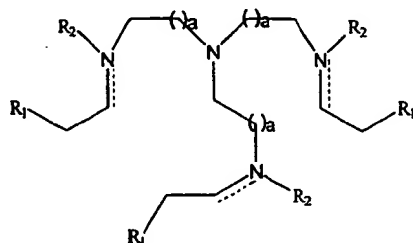


(I)

where each a independently represents an integer between 1 and 3, each R independently represents hydrogen or hydroxy and each R₁ independently represents a carboxylate, phosphate, thioacid, thiol, amino alkoxide or hydroxy group;

20

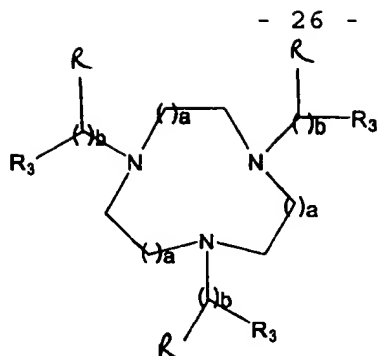
25



(II)

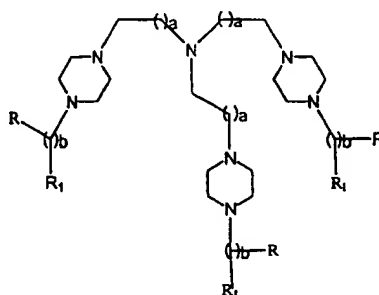
where a and R₁ are as hereinbefore defined and each R₂ independently represents hydrogen, C₁₋₆ alkyl or aryl, with the proviso that R₂ is absent when the double bond is present on the same nitrogen;

35



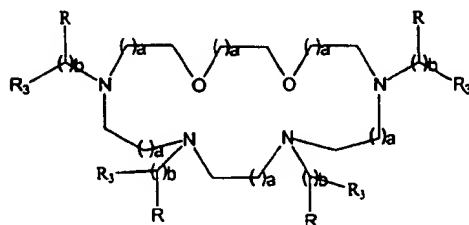
(III)

where a , R and R_2 are as hereinbefore defined, b is an integer between 0-3 and each R_3 independently represents R_1 , $NR-NR_2-COO^\ominus$, or $N=N-COO^\ominus$ when b is positive or each R_3 independently represents $N=CH-COO^\ominus$ or $NR_2-CH_2-COO^\ominus$;



(IV)

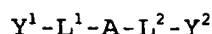
where a , b , R and R_1 are as hereinbefore defined;



(V)

where a , b , R and R_3 are as hereinbefore defined;

- 27 -



5

(VI)

where A is N, CR₄, P, P=O, *cis,cis,cis*-1,3,5-trisubstituted-cyclohexane or an N,N',N"-trisubstituted-triaza 9 to 14 membered macrocyclic ring;

10 L¹, L², L³ are linker groups which are independently chosen from C₁₋₄ alkylene, C₄₋₈ cycloalkylene or C₄₋₈ o-arylene;

Y¹, Y², Y³ are independently chosen from -NH₂,
15 -B(=O)OZ, -N=CR₅-B(=O)OZ, -NR₅-CR₆-B(=O)OZ, -N[CR₆-B(=O)Q]₂ and -O-CR₆-B(=O)OZ where B is C or PR₆, each Q is independently -OZ or -NR₆, and Z is H or a counter-ion;

each R₄ and R₅ group is independently chosen from H, C₁₋₅ alkyl, C₁₋₅ alkoxyalkyl, C₁₋₅ hydroxyalkyl,
20 C₁₋₅ aminoalkyl, C₅₋₁₀ aryl or C₁₋₆ fluoroalkyl;

R₆ is OH, C₁₋₆ alkyl, C₁₋₆ alkoxyalkyl, C₁₋₆ fluoroalkyl, C₁₋₁₀ alkoxy or C₅₋₁₀ aryl;

with the proviso that at least one of Y¹, Y² and Y³ is -N=CR₅-B(=O)OZ.

25

13. A method as claimed in any preceding claim wherein said agent is conjugated to a biological vector capable of targeting said agent to a desired region of the body.

30 14. A method as claimed in claim 13 wherein said biological vector is selected from the group consisting of an antibody, an antibody fragment and an oligopeptide binding motif.

35 15. A method as claimed in any preceding claim wherein conversion between said first and second oxidation states is effected *in vivo* by a localised normal or

- 28 -

abnormal biological process, by an administered chemical agent or by illumination of said agent with light.

5 16. A method as claimed in claim 15 wherein conversion between said first and second oxidation states is effected in vivo by the presence or absence of oxygen or of oxidation or reduction promoting agents, from a change in temperature or as a result of an increase or decrease in pH at the target site, or as a result of the
10 presence of a specific enzyme.

17. A method as claimed in claim 15 wherein said chemical agent is a redox reagent capable of delivery to or accumulation at a desired target site within the
15 body.

18. A method as claimed in claim 15 wherein conversion between said first and second oxidation states is effected by application of light having a wavelength of
20 from 600 to 1300 nm.

19. An MR contrast agent composition comprising as an MR contrast agent a physiologically tolerable lanthanide compound or salt thereof having first and second
25 oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur,
30 together with an optionally encapsulated physiologically tolerable trigger substance capable of converting said contrast agent between said first and second oxidation states.

35 20. A composition as claimed in claim 19 wherein said trigger substance is an enzyme, a redox agent or a free radical scavenger.

- 29 -

21. The use of a physiologically tolerable MR contrast agent substance comprising a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to a method as claimed in any one of claims 1 to 18.

22. Use as claimed in claim 21 for the manufacture of a diagnostic contrast medium for use in a method of detecting malignant melanoma, squamous cell carcinoma, sarcomas or adenocarcinomas.

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 49/00	A2	(11) International Publication Number: WO 00/24429 (43) International Publication Date: 4 May 2000 (04.05.00)
(21) International Application Number: PCT/GB99/03488 (22) International Filing Date: 22 October 1999 (22.10.99) (30) Priority Data: 9823175.6 22 October 1998 (22.10.98) GB (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/107212 (CIP) Filed on 5 November 1998 (05.11.98) (71) Applicant (for all designated States except US): NYCOMED IMAGING AS [NO/NO]; Nycoveien 1-2, N-0401 Oslo (NO). (71) Applicant (for GB only): GOLDING, Louise [GB/GB]; 179 Queen Victoria Street, London EC4V 4EL (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): KELLAR, Kenneth [US/US]; Nycomed Inc., 466 Devon Park Drive, P.O. Box 6630, Wayne, PA 19087-8630 (US).		(74) Agents: GOLDING, Louise et al.; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB). (81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: COMPOUND (57) Abstract The invention relates to the use as a contrast agent in MR imaging of a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible <i>in vivo</i> from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Compound

5 This invention relates to compounds useful as contrast agents in magnetic resonance imaging and to methods of imaging using such compounds.

10 Magnetic resonance (MR) imaging is a well established imaging modality in which the image is derived from the intensity of the nmr signal from protons (usually water protons) in the subject under study. Because most tissue has an approximately 80% water content, contrast in MR imaging is attained by the application of pulse sequences that reveal differences in the relaxation times (T_1 and T_2) of the tissues. As
15 with other diagnostic imaging modalities such as CT and ultrasound, contrast agents may be used in MR imaging procedures to enhance contrast in the images produced, e.g. to allow clearer differentiation between different tissue types or between healthy and non-healthy tissue.
20 In MR imaging, the contrast agents conventionally are chelated paramagnetic species (e.g. Gd DTPA, Gd DTPA-BMA and Gd HP-DO3A, available commercially under the trade names Magnevist, Omniscan and Pro-Hance), which achieve contrast enhancement because of their relaxivities,
25 their ability to decrease the relaxation times of water protons.

A proposal has been made, in WO96/38184, that "triggered" paramagnetic metal ion complexes be used as MR contrast agents. As described in WO96/38184, the
30 trigger mechanism has the paramagnetic complex being "turned on" as an MR contrast agent by the presence of a target substance which interacts with the agent complexing the paramagnetic metal ion so as to free an inner sphere coordination site and allow water molecule
35 exchange to take place at the freed-up site. In the absence of the target substance, the complexed paramagnetic metal ion has no inner sphere coordination

- 2 -

sites available for water molecule exchange and in this state the contrast agent is considered to be turned off.

This concept of a triggered MR contrast agent however has a major defect which will hinder practical application of the concept. Thus in the "turned off" state the complex will still function fairly effectively as an MR contrast agent since both inner-sphere and outer-sphere water coordination contributes to the agent's relaxivity. The inventors of WO96/38184 indirectly acknowledge this drawback when they refer to the degree of change in MR signal that is sufficient to be detectable in the image as being as low as 2 to 5%, well below the conventionally accepted threshold of 10% (see for example Chem. Rev. 87: 901-927 (1987)). The relaxivity of the gadolinium chelates of WO96/38184 will be reduced by about one half (but not eliminated) if inner sphere coordination of water is prevented. Thus the triggered agents of WO96/38184 are not so much switched off as dimmed by about half by the absence of the target substance. Accordingly the selectivity and sensitivity desired by the authors is not possible due to the unavoidable outer-sphere contribution.

It has since been proposed by the applicants in WO98/47539 that triggered MR imaging of contrast agents may be achieved significantly more efficiently by using the "target substance" to change the contrast agent between states in which the relaxivity (r_1) differs by a factor of at least 5. This is achieved either by switching to a lower relaxivity state with little or no relaxivity or alternatively by switching on/off an inner sphere deriving relaxivity which is significantly higher than (e.g. 5 times or greater than) the outer sphere deriving component of the relaxivity.

Certain contrast agents which have now been found to be particularly suitable for use in "triggered" MR imaging techniques are those comprising lanthanide compounds which can be switched between first and second

- 3 -

oxidation states differing in relaxivity by a factor of 5 or more, preferably 10 or more, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low
5 relaxivity state approaches zero. "Triggered" MR imaging is achieved using such agents as a result of a redox reaction.

Thus viewed from one aspect the invention provides a method of generating a contrast enhanced image of a
10 human or non-human (preferably mammalian) animal subject which comprises administering to said subject an effective amount of a magnetic resonance imaging contrast agent and generating an image of at least part of said subject containing said agent, wherein said
15 agent comprises a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, preferably at least 10, but can be much higher, e.g. at least 20, at least 100 or even
20 significantly larger if the relaxivity of the low relaxivity state approaches zero, and which is convertible *in vivo* from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or
25 does not occur.

In the method of the invention the change between high and low relaxivity states is effected as a change in the oxidation state of the lanthanide metal in the contrast agent between higher and lower relaxivity
30 states. In this regard, the means for effecting the change between higher and lower relaxivity states may be localised normal or abnormal biological activity, an administered chemical agent or an applied physical means (e.g. illumination with light).

35 The change in oxidation state may give rise to a change in relaxivity in a number of ways, e.g. as a result of a change from a paramagnetic to a diamagnetic

- 4 -

state, from a diamagnetic to a paramagnetic state, or from one paramagnetic state to another. Conveniently, the change in relaxivity of the contrast agent is effected as a change from one paramagnetic state to another, e.g. from a non-spherically symmetric electronic ground state to a spherically symmetric electronic ground state, or a change from a non-spherically symmetric electronic ground state to a spherically symmetric excited state. The non-spherically symmetric state will have a much lower associated relaxivity than the spherically symmetric state and accordingly the contrast difference between the "on" and "off" states of the switchable agent is large.

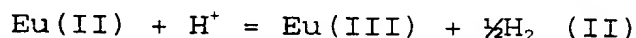
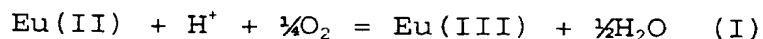
Preferably, the contrast agent for use in the method of the invention is a chelate complex of a lanthanide metal ion in which the chelated metal ion is capable of redox conversion from one oxidation state to another (one or both of which are paramagnetic). On/off switching by a redox reaction may occur either as a result of oxidation or reduction of the chelated metal ion. Depending on the particular lanthanide metal present, its initial oxidation state and the nature of the complexing agent, this may bring about either a decrease or increase in relaxivity of the contrast agent.

Preferred contrast agents for use in the invention are those in which the "on position" corresponds to a state in which the relaxivity is as high as possible and in which the "off position" corresponds to a state in which the relaxivity is as low as possible, preferably close to zero. In this regard, contrast agents comprising Europium compounds, in particular chelate complexes of Europium, which are activated by switching between the II and III oxidation states, e.g. by biological activity or by redox reagents are particularly preferred for use in the method of the

- 5 -

invention.

Due to a half filled 4f shell, Eu(II) complexes have a spherically-symmetric electronic ground state ($^8S_{7/2}$) and therefore have long electron spin relaxation times and particularly high relaxivities. Eu(III) complexes, on the other hand, have a 7F_0 electronic ground state and very short electronic relaxation times. Eu(III) is only paramagnetic because excited states must be considered, but these states are not spherically symmetric. Consequently, electronic relaxation times are very short and relaxivities are essentially zero. Oxidation of Eu(II) to Eu(III) thus causes a substantial loss of relaxivity which is readily detectable as a marked change in MR signal intensity. The transition from Eu(II) to Eu(III) thus provides a highly sensitive "on-off" switch. Moreover, the transition from Eu(II) to Eu(III) is particularly sensitive to oxygen concentration and pH:



Equation (I) is dominant when oxygen is present.

Suitable complexing agents for use in the invention are those which present the lanthanide metal, in particular Europium, in a biotolerable form, e.g. a polyaminopolyacid chelating agent of the type well known for MR agents and radiopharmaceuticals, for example DTPA, EDTA, DTPA-BMA, DO3A, DOTA, HP-DO3A, TMT, DPDP, etc. In this regard the reader is referred to the patent publications of metal chelates from Schering, Nycomed, Salutar, Bracco, Mallinckrodt, Guerbet, Sterling Winthrop, etc. Examples include US-A-4647447, US-A-5362475, US-A-5534241, US-A-5358704, US-A-5198208, US-A-4963344, EP-A-230893, EP-A-130934, EP-A-606683, EP-A-438206, EP-A-434345, WO 97/00087, WO 96/40274, WO 96/30377, WO 96/28420, WO 96/16678, WO 96/11023,

WO 95/32741, WO 95/27705, WO 95/26754, WO 95/28967,
WO 95/28392, WO 95/24225, WO 95/17920, WO 95/15319,
WO 95/09848, WO 94/27644, WO 94/22368, WO 94/08624,
WO 93/16375, WO 93/06868, WO 92/11232, WO 92/09884,
5 WO 92/08707, WO 91/15467, WO 91/10669, WO 91/10645,
WO 91/07191, WO 91/05762, WO 90/12050, WO 90/03804,
WO 89/00052, WO 89/00557, WO 88/01178, WO 86/02841 and
WO 86/02005.

Thus appropriate complexing agents include
10 macrocyclic chelants having an open coordination site
for water, e.g. porphyrin-like molecules and the
pentaaza macrocyclic ligands of Zhang et al (Inorg.
Chem. 37(5):956-963, 1998), phthalocyanines, crown
ethers e.g. nitrogen crown ethers such as the
15 sepulchrates, cryptates etc., hemin (protoporphyrin IX
chloride) and heme (available from Porphyrin Products,
Inc. of Logan, Utah, USA) and chelants having a square-
planar symmetry. Alternatively, the complexing agent
may comprise a polyacid ligand capable of protonating a
20 coordinating group thereby freeing up a coordination
site for water molecules at a particular pH.

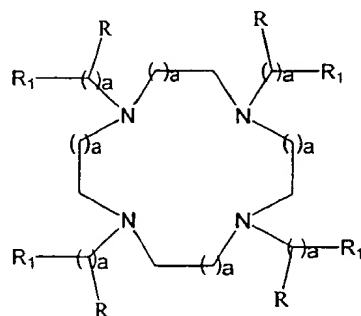
Other complexing agents of use according to the
invention include polyoxadiazamacrobicyclic ligands
("cryptands") known to form stable coordination
25 compounds ("cryptates") with several lanthanide metal
ions, in particular with Europium (see J. Am Chem. Soc.
102(7): 2278-2285, 1980). In this regard, the (2.2.1),
(2.2.2) and (2₈.2.1) cryptands are particularly suitable
for use in the invention [the numerals within the
30 parentheses refer to the number of oxygen atoms in the
polyether bridges joining the nitrogen bridgeheads in
the bicyclic molecule. Thus, (2.2.1) cryptand =
4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane
and (2.2.2) cryptand = 4,7,13,16,21,24-hexaoxa-1,10-
35 diazabicyclo [8.8.8]hexacosane. The ligand (2₈.2.1) is
similar to (2.2.1) except that one of the central
dioxyethylene groups is replaced by the analogous

- 7 -

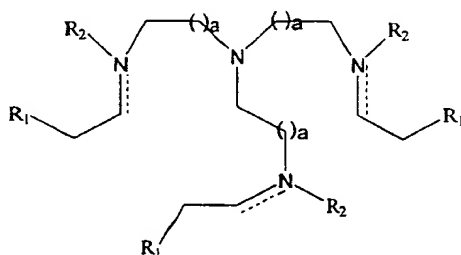
catechol].

Particular Europium compounds for use in the invention include the following cryptates: $\text{Eu}^{\text{II}}(2.2.1)$, $\text{Eu}^{\text{II}}(2_{\beta}.2.1)$, $\text{Eu}^{\text{II}}(2.2.2)$ and the corresponding Eu^{III} complexes, $\text{Eu}^{\text{III}}(2.2.1)$, $\text{Eu}^{\text{III}}(2_{\beta}.2.1)$ and $\text{Eu}^{\text{III}}(2.2.2)$.

Suitable complexing agents also include ligands of formula (I)

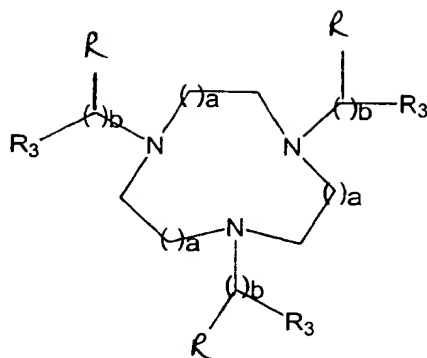


where each a independently represents an integer between 1 and 3, preferably 1, each R independently represents hydrogen or hydroxy and each R_1 independently represents a carboxylate, phosphate, thioacid, thiol, amino alkoxide or hydroxy group, preferably carboxylate; formula (II)



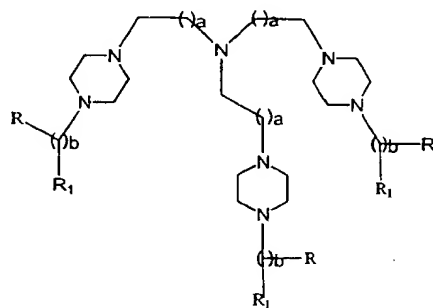
where a and R_1 are as hereinbefore defined and each R_2 independently represents hydrogen, C_{1-6} alkyl, e.g. methyl or isopropyl, aryl, e.g. phenyl, with the proviso that R_2 is absent when the double bond is present on the same nitrogen;

formula (III)

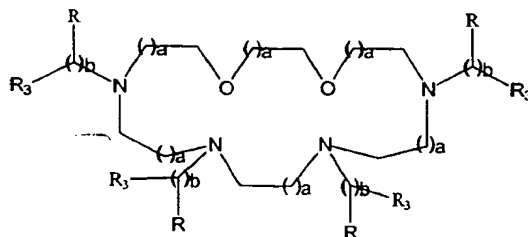


where a , R and R_2 are as hereinbefore defined, b is an integer between 0-3 and each R_3 independently represents R_1 , $NR-NR_2-COO^\ominus$, or $N=N-COO^\ominus$ when b is positive or each R_3 independently represents $N=CH-COO^\ominus$ or $NR_2-CH_2-COO^\ominus$;

formula (IV)

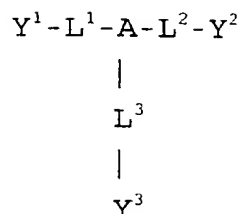


where a , b , R and R_1 are as hereinbefore defined; and formula (V)



where a , b , R and R_3 are as hereinbefore defined. Also of use are complexing agents of formula (VI)

- 9 -



5

where A is N, CR₄, P, P=O, *cis,cis,cis*-1,3,5-trisubstituted-cyclohexane or an N,N',N''-trisubstituted-triaza 9 to 14 membered macrocyclic ring;

L¹, L², L³ are linker groups which are independently chosen from C₁₋₄ alkylene, C₄₋₈ cycloalkylene or C₄₋₈ o-arylene;

15

Y¹, Y², Y³ are independently chosen from -NH₂, -B(=O)OZ, -N=CR₅-B(=O)OZ, -NR₅-CR₆-B(=O)OZ, -N[CR₆-B(=O)Q]₂ and -O-CR₆-B(=O)OZ where B is C or PR₆, each Q is independently -OZ or -NR₆, and Z is H or a counter-ion;

20

each R₄ and R₅ group is independently chosen from H, C₁₋₅ alkyl, C₁₋₅ alkoxyalkyl, C₁₋₅ hydroxyalkyl, C₁₋₅ aminoalkyl, C₅₋₁₀ aryl or C₁₋₆ fluoroalkyl;

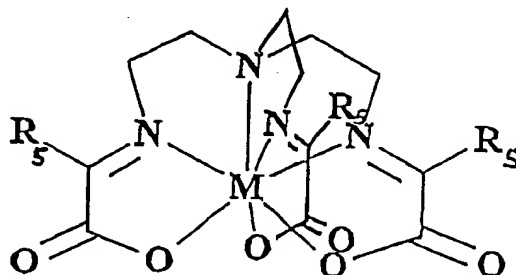
R₆ is OH, C₁₋₆ alkyl, C₁₋₆ alkoxyalkyl, C₁₋₆ fluoroalkyl, C₁₋₁₀ alkoxy or C₅₋₁₀ aryl;

25

with the proviso that at least one of Y¹, Y² and Y³ is -N=CR₅-B(=O)OZ.

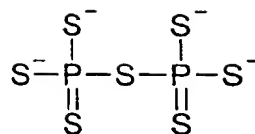
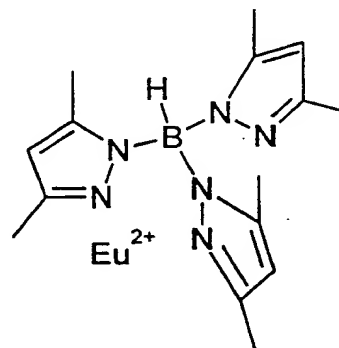
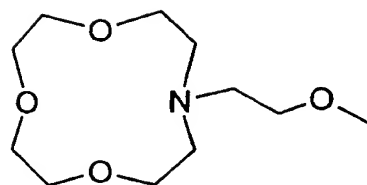
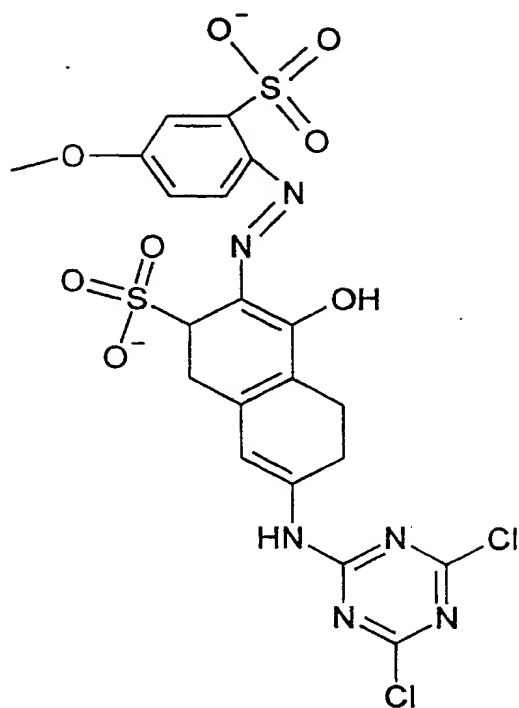
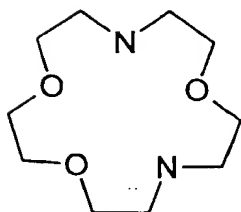
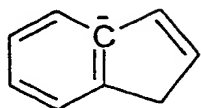
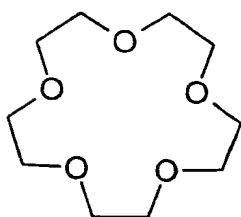
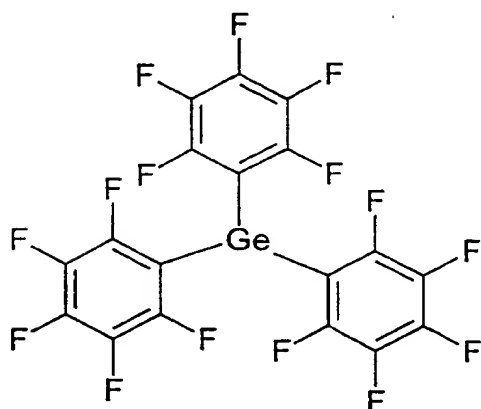
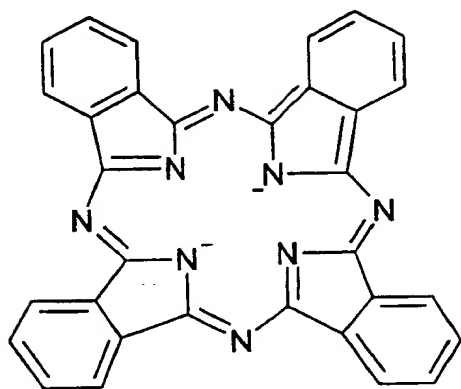
For example

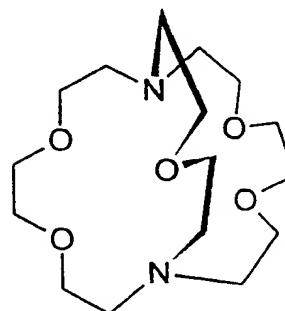
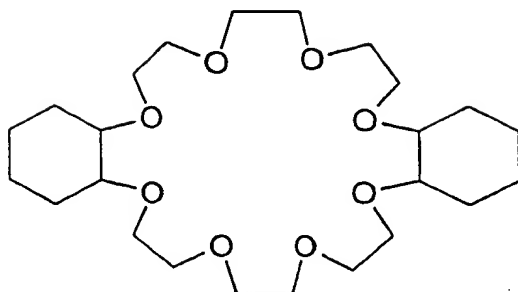
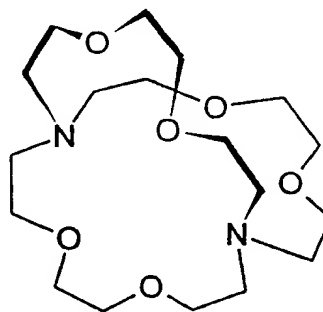
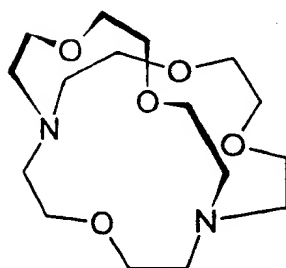
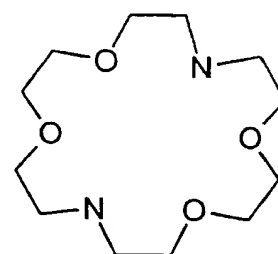
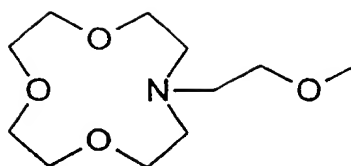
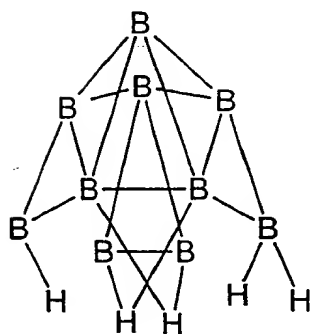
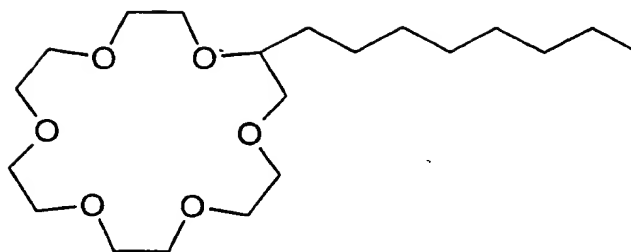
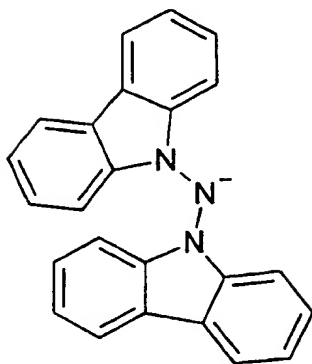
30



35

Specific complexing agents of use according to the invention include





Certain complexing agents may affect the redox couple and may stabilise the metal in a higher or lower oxidation state. The complexing agent may also significantly affect the biodistribution. For example depending on the charge on the metal ion and the degree of ionisation of the complexing agent, the metal complexes of use in the present invention may be charged or neutral. Neutral complexes, which do not carry highly hydrophilic substituents may be sufficiently lipophilic to cross lipid membranes such as cell membranes or the blood-brain barrier. Lipophilicity can be readily adjusted by varying the nature of the complexing agent and will be readily achieved by the skilled person. The change in relaxivity in switching between different oxidation states may be further enhanced by having as the first oxidation state a very high relaxivity compound, such as a polymeric chelate of the lanthanide metal ion, or a rigid paramagnetic polychelate of the lanthanide metal ion, e.g. a vector targeted lanthanide chelate or polychelate, or a dendrimeric chelate of a lanthanide metal ion such as described in WO93/06868 with a short linkage or a hydrophobic linkage between dendrimeric branching sites. Further enhancement of the sensitivity of the "on-off" switch may be achieved by having as the second oxidation state a very low relaxivity compound. This may be achieved, for example, by having as the low relaxivity compound the same material with the lanthanide metal present in the second oxidation state and (i) with water coordination sites reversibly blocked by an enzymically removable blocking group (e.g. as described in WO96/38184) and/or (ii) with a targeting vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif such as RGD) which in that state is not bound to its intended substrate.

Thus the switching between low and high relaxivity states may be further enhanced by binding of the

targeting vector or displacement of the blocking groups or alternatively by interaction at the target site to change a high relaxivity conformation into a low relaxivity conformation, or vice versa. Such
5 conformational changes can be achieved for the compounds of PCT/GB96/01308 by changing the chemical nature of their immediate environment (e.g. by the presence or addition of urea).

Preferably, the contrast agent for use in
10 accordance with the invention may be further conjugated to a macromolecule. In this way the relaxivity of the contrast agent is further increased thereby enhancing the sensitivity of the "on-off" switch between relaxivity states. Examples of suitable macromolecules
15 include proteins and polymers, e.g. that prepared in accordance with Example 2 of WO98/10797, and macrostructures such as liposomes in which the chelate is bound to the outer surface.

The means by which conversion from one oxidation
20 state to another may be achieved may be a biological process or malfunction. Accordingly, the method of the invention finds application in methods of "functional" MR imaging capable of providing vital information relating to the functioning of particular parts of the
25 body. For example, the method of the invention can be used to identify parts of the body which may be functioning abnormally, e.g. as a result of disease.

Transition between the "on" and "off" states may, for example, result from the presence or absence of
30 oxygen or of oxidation or reduction promoting agents, from a change in temperature or as a result of an increase or decrease in pH at the target site, or as a result of the presence of a specific enzyme. For
example, in the case of an "on-off" system, the MR image
35 will appear bright unless there is a specific condition present such as oxygen deficiency which causes switching of the contrast agent to the lower relaxivity state and

a corresponding reduction of image intensity to that which would be expected in the absence of the contrast agent.

Alternatively the means for conversion may be a
5 chemical agent administered to the subject, e.g. a redox reagent capable of delivery to or accumulation at a desired target site within the body, or designed for release at such a site for example a tumour or oedema. In some cases, activation of the agent may involve
10 application of light, preferably with a wavelength of from 600 to 1300 nm in order to minimise absorption by the body.

As mentioned above, in one aspect of the invention the relaxivity of the contrast agent may be switched as
15 a result of a change in pH. Contrast agents for use in the method of the invention may thus be used to detect areas of the body which are acidic or basic due to physiological or disease processes. Typically, they may be used to detect regions of pH of about 4 to ~5.5
20 within the body by appropriate selection as the contrast agent of a substance having a pKa value above or below a predictable threshold.

For example, many tumors exhibit a lower extracellular pH, e.g. as low as 5.5, typically between
25 ~5.5 and ~7.7. This is a result of decreased vascular perfusion resulting in chronic hypoxia and increased lactic acid levels, exacerbated by the typically higher metabolic rate of cancerous cells.

Certain metal complexes undergo more rapid
30 hydrolysis at lower pH, e.g. those of formula (VI) above as described in WO98/39288 which is herein incorporated by reference. Due to the rapid hydrolysis, metal ions are selectively trapped in the area of low pH allowing targeting of the metal ion to certain areas of the body.

35 On the other hand, necrotic areas within tumors may exhibit a higher, more basic, pH. Acidic tumor types which may be detected using the method of the invention

include malignant melanoma, squamous cell carcinoma, sarcomas and adenocarcinomas (see Thistlewaite et al., Int. J. Radiation Oncology Biol. Phys. 11: 1647-1652, 1985).

5 Osteoporosis is a degenerative bone disorder. During the physiological process of bone resorption osteoclasts excavate small pits throughout the bone, creating a zone of reduced pH between the osteoclast and the bone tissue. pH values as low as 4.0 have been
10 measured in the active erosion zones (see Silver et al., Cell Res. 175: 266-267, 1988). Effective imaging of this erosion zone using the method of the invention may be used to provide vital information regarding the effectiveness of therapies used in the treatment of
15 osteoporosis. In this way, the clinician may readily determine the therapeutic effect of a given drug and use the information either to continue therapy or to change therapies.

 Measurement of local osteoclastic activity using
20 the method of the invention may also be used to evaluate other bone remodelling activities such as the repair of fractures, the treatment of Paget's disease or to evaluate the extent of expanding lesions in bone, such as tumors, in which resorption may take place at the
25 bone surface in contact with the lesion.

 In the method of the invention, the region in which the conversion from first to second relaxivity state occurs will preferably be identified, e.g. by comparison with a "native" image in the collection of which the
30 means for conversion has not been administered or activated or with a comparison body site in which the biological process responsible for the conversion does not occur.

 The contrast agents for use in the method of the
35 invention, in particular those comprising chelating agents, may if desired be conjugated to biological vectors so as to target actively or passively to the

desired regions of the body. Conjugation of metal chelates to targeting vectors is discussed for example in WO93/21957 and US-A-5595725 (Schering).

Where the targeting vector is such as to bind the agent to a target site, relaxivity will be increased as a result and in one embodiment of the invention the triggering of enhanced relaxivity may be achieved by a combination of the freeing up of a coordination site according to WO96/38184 and binding to a larger structure, e.g. a cell wall or the wall of a body duct using a vector (e.g. an antibody, antibody fragment, or an oligopeptide binding motif (such as RGD) conjugated to a compound according to WO96/38184.

The contrast agent for use in the method of the invention may be for example a complex of a lanthanide metal ion having first and second oxidation states and, in the high relaxivity state, at least one open coordination site for the exchange of water molecules. Such agents are capable of switching between first and second relaxivity states as a result of a change in pH. In the case of Eu(II), a change in pH may be sufficient to alter the chelate so that this becomes very sensitive to oxygen concentration and so able to make the transition to Eu(III).

For administration into the GI tract, it may be unnecessary to chelate the metal and thus for this route simple salts, e.g. chlorides, may be used.

The contrast agent may be administered by any convenient route, eg. topical, transdermal, nasal, sublingual, oral, rectal, by direct instillation into an externally voiding body cavity (eg. lungs, uterus, GI tract and bladder), or subcutaneously, intramuscularly, interstitially or into the vasculature, eg. by injection or infusion. In general administration into the vasculature or into the GI tract will be preferred routes.

For administration, the contrast agent may be

formulated together with appropriate conventional pharmaceutically acceptable carrier or excipients, such as liquid carriers (eg. saline or water for injections), pH and osmolality regulators, stabilizers, viscosity
5 modifiers, surfactants, bulking agents, skin penetration agents, flavourings, solid or semi-solid carriers (eg. hydrophilic gels), aerosol dispersants, etc.

The dose of contrast agent required will depend on the species and condition under study, the selected
10 contrast agent, and the administration route. However in general doses for i.v. administration will normally be in the range 0.001 to 5.0 mmol paramagnetic centre/kg bodyweight (where by paramagnetic centre is meant a metal atom which is or becomes paramagnetic).

15 If a chemical agent (a "trigger", for example an enzyme, a redox agent or a free radical scavenger) is administered to trigger the conversion between states of different relaxivity, then this can be administered together with the contrast agent or separately, eg.
20 before or after or even simultaneously in the event that the administration site is different. If coadministered, then the trigger may be formulated to contact the contrast agent only after delivery or on reaching the target site. Thus for example it may be
25 encapsulated in a matrix or membrane (e.g. a vesicle membrane) which breaks down or is broken down at the desired target site. If the method of the invention is used intraoperatively, e.g. to highlight damage to a particular tissue or organ or to delineate a mass to be
30 removed, the trigger may be applied to the operating site during the operation so as to switch on the contrast agent at the cutting site. In this event the trigger may be a chemical agent as discussed above, the air, or an applied physical stimulus.

35 The compositions containing both the contrast agent and a chemical trigger are themselves new and form a further aspect of the invention. Viewed from this

aspect the invention provides an MR contrast agent composition comprising as an MR contrast agent a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, preferably at least 10, but can be much higher, e.g. at least 20, at least 100 or even significantly larger if the relaxivity of the low relaxivity state approaches zero, and which is convertible *in vivo* from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, together with an optionally encapsulated physiologically tolerable trigger substance capable of converting said contrast agent between said first and second oxidation states.

Viewed from a further aspect the invention also provides the use of a physiologically tolerable MR contrast agent substance comprising a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible *in vivo* from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to the method of the invention.

All documents referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the accompanying non-limiting Examples.

Example 1

This example involves the use of a Eu(II)-chelate for detecting a low oxygen concentration in a tumor. An Eu(II)-chelate would be injected intravenously and a T₁-

weighted pulse sequence would be used to obtain the images for a variety of times post injection of the Eu(II)-chelate. In the first image obtained at a time immediately after injection, the blood pool containing the Eu(II)-chelate would appear as being bright (high signal intensity). As time elapses, the Eu(II)-chelate will distribute into tissue, and the Eu(II) will oxidise to Eu(III) at a rate that depends on the local oxygen concentration. As a result of the oxidation, the signal intensity of images obtained at later times of regions containing the Eu-chelate will decrease as more Eu(III) is formed. The signal intensity will decrease more rapidly with time in regions of high oxygen concentration. Therefore, regions of low oxygen concentration will have a signal intensity that decreases more slowly with time, and these regions could eventually appear as bright spots on the images. Such a sensitivity to oxygen concentration could prove very useful in the characterisation of tumors, for example. The sensitivity to oxygen concentration could also prove useful in the evaluation of cardiac tissue and possibly stroke as well. However, in the evaluation of stroke and/or brain perfusion, it may be useful to use a T₂-weighted pulse sequence.

Example 2

This example involves the use of an Eu(II)-chelate conjugated to a macromolecule, designed for characterising the oxygen content of a tumor. As an example, the Eu(II)-chelate-macromolecule complexes are similar to the Gd(III)-chelate-macromolecule complexes described in T.S. Desser, K.I. Rubin, H.H. Muller, F. Qing, S. Khodar, G. Zanazzi, S.W. Young, D.L. Ladd, J.A. Wellons, K.E. Kellar, J.L. Toner, R.A. Snow, "Dynamics of Tumor Imaging with Gd-DTPa-Polyethylene Glycol Polymers: Dependence on Molecular Weight" Journal of

Magnetic Resonance Imaging 4, 467-472 (1994), where
Eu(II) takes the place of Gd(III). In this work,
macromolecular complexes have been shown to have an
extended lifetime in the blood pool as a result of
5 conjugating the metal chelate to a polymer, and this
increased lifetime enables the complexes to be taken up
by tumors. However, unlike their Gd(III)-containing
counterparts, the Eu(II)-containing macromolecular
complexes will be sensitive to the oxygen content of the
10 tumors as described in Example 1 above.

Claims:

1. A method of generating a contrast enhanced image of a human or non-human animal subject which comprises
5 administering to said subject an effective amount of a magnetic resonance imaging contrast agent and generating an image of at least part of said subject containing said agent, wherein said agent comprises a physiologically tolerable lanthanide compound or salt
10 thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible *in vivo* from said first to said second oxidation state whereby contrast is enhanced in a body region in which conversion to said second state
15 does or does not occur.
2. A method as claimed in claim 1 wherein said agent comprises a physiologically tolerable lanthanide compound or salt thereof having first and second
20 oxidation states which differ in relaxivity by a factor of at least 10.
3. A method as claimed in claim 1 wherein said agent comprises a physiologically tolerable lanthanide
25 compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 20.
4. A method as claimed in claim 1 wherein said agent
30 comprises a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 100.
5. A method as claimed in any one of claims 1 to 4
35 wherein the change between said first and said second oxidation states is effected as a change from a

paramagnetic to a diamagnetic state, as a change from a diamagnetic to a paramagnetic state, or as a change between two paramagnetic states of the lanthanide metal ion.

5

6. A method as claimed in claim 5 wherein said change between two paramagnetic states is effected as a change from a non-spherically symmetric electronic ground state to a spherically symmetric electronic ground state, or a
10 change from a non-spherically symmetric electronic ground state to a spherically symmetric excited state.

7. A method as claimed in any preceding claim wherein said agent is a chelate complex of a lanthanide metal
15 ion, or a physiologically tolerable salt thereof.

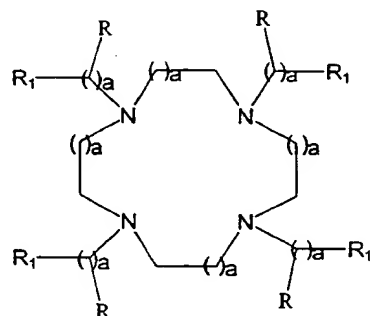
8. A method as claimed in any preceding claim wherein said agent is a Europium compound, preferably a chelate complex of Europium or a physiologically tolerable salt
20 thereof.

9. A method as claimed in claim 8 wherein said Europium compound is activated by switching between the II and III oxidation states of the metal ion.
25

10. A method as claimed in any one of claims 7 to 9 wherein said chelate complex is a complex of a chelant selected from the group consisting of DTPA, EDTA, DTPA-BMA, DO3A, DOTA, HP-DO3A, TMT and DPDP.
30

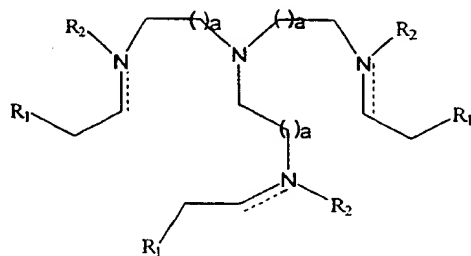
11. A method as claimed in any one of claims 7 to 9 wherein said chelate complex is a complex of a chelant selected from the group consisting of porphyrins and porphyrin-like molecules, phthalocyanines, crown ethers, hemin, heme, chelants having a square planar symmetry, cryptands and cryptates.
35

12. A method as claimed in any one of claims 7 to 9 wherein said chelate complex is a complex of a chelant selected from compounds of formulae (I), (II), (III), (IV), (V) and (VI):



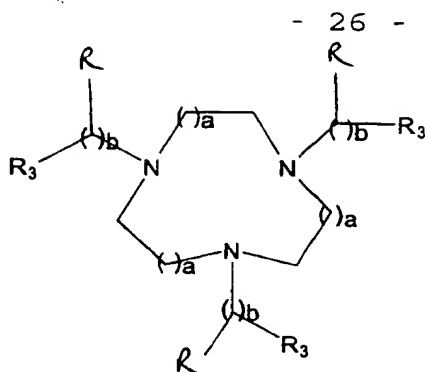
(I)

where each a independently represents an integer between 1 and 3, each R independently represents hydrogen or hydroxy and each R_1 independently represents a carboxylate, phosphate, thioacid, thiol, amino alkoxide or hydroxy group;



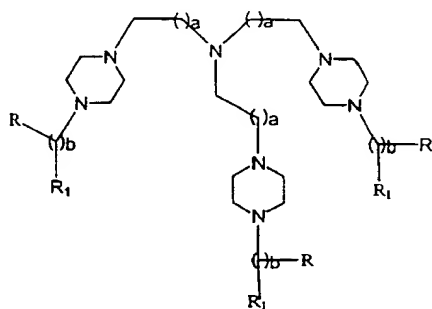
(II)

where a and R_1 are as hereinbefore defined and each R_2 independently represents hydrogen, C_{1-6} alkyl or aryl, with the proviso that R_2 is absent when the double bond is present on the same nitrogen;



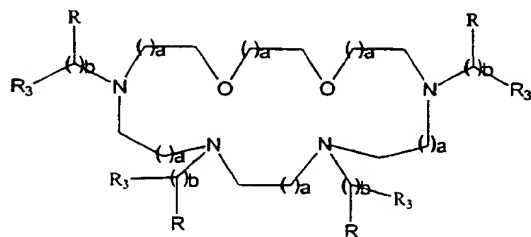
(III)

where a , R and R_2 are as hereinbefore defined, b is an integer between 0-3 and each R_3 independently represents R_1 , $NR-NR_2-COO^\ominus$, or $N=N-COO^\ominus$ when b is positive or each R_3 independently represents $N=CH-COO^\ominus$ or $NR_2-CH_2-COO^\ominus$;



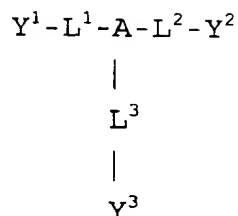
(IV)

where a , b , R and R_1 are as hereinbefore defined;



(V)

where a , b , R and R_3 are as hereinbefore defined;



5

(VI)

where A is N, CR₄, P, P=O, *cis,cis,cis*-1,3,5-trisubstituted-cyclohexane or an N,N',N''-trisubstituted-triaza 9 to 14 membered macrocyclic ring;

L¹, L², L³ are linker groups which are independently chosen from C₁₋₄ alkylene, C₄₋₈ cycloalkylene or C₄₋₈ o-arylene;

Y¹, Y², Y³ are independently chosen from -NH₂, -B(=O)OZ, -N=CR₅-B(=O)OZ, -NR₅-CR₆-B(=O)OZ, -N[CR₆-B(=O)Q]₂ and -O-CR₆-B(=O)OZ where B is C or PR₆, each Q is independently -OZ or -NR₆, and Z is H or a counter-ion;

each R₄ and R₅ group is independently chosen from H, C₁₋₅ alkyl, C₁₋₅ alkoxyalkyl, C₁₋₅ hydroxyalkyl, C₁₋₅ aminoalkyl, C₅₋₁₀ aryl or C₁₋₆ fluoroalkyl;

R₆ is OH, C₁₋₆ alkyl, C₁₋₆ alkoxyalkyl, C₁₋₆ fluoroalkyl, C₁₋₁₀ alkoxy or C₅₋₁₀ aryl;

with the proviso that at least one of Y¹, Y² and Y³ is -N=CR₅-B(=O)OZ.

25

13. A method as claimed in any preceding claim wherein said agent is conjugated to a biological vector capable of targeting said agent to a desired region of the body.

30

14. A method as claimed in claim 13 wherein said biological vector is selected from the group consisting of an antibody, an antibody fragment and an oligopeptide binding motif.

35

15. A method as claimed in any preceding claim wherein conversion between said first and second oxidation states is effected *in vivo* by a localised normal or

abnormal biological process, by an administered chemical agent or by illumination of said agent with light.

16. A method as claimed in claim 15 wherein conversion
5 between said first and second oxidation states is
effected *in vivo* by the presence or absence of oxygen or
of oxidation or reduction promoting agents, from a
change in temperature or as a result of an increase or
10 decrease in pH at the target site, or as a result of the
presence of a specific enzyme.

17. A method as claimed in claim 15 wherein said
chemical agent is a redox reagent capable of delivery to
or accumulation at a desired target site within the
15 body.

18. A method as claimed in claim 15 wherein conversion
between said first and second oxidation states is
effected by application of light having a wavelength of
20 from 600 to 1300 nm.

19. An MR contrast agent composition comprising as an
MR contrast agent a physiologically tolerable lanthanide
compound or salt thereof having first and second
25 oxidation states which differ in relaxivity by a factor
of at least 5, and which is convertible *in vivo* from
said first to said second oxidation state whereby
contrast is enhanced in a body region in which
conversion to said second state does or does not occur,
30 together with an optionally encapsulated physiologically
tolerable trigger substance capable of converting said
contrast agent between said first and second oxidation
states.

20. A composition as claimed in claim 19 wherein said
35 trigger substance is an enzyme, a redox agent or a free
radical scavenger.

21. The use of a physiologically tolerable MR contrast agent substance comprising a physiologically tolerable lanthanide compound or salt thereof having first and second oxidation states which differ in relaxivity by a factor of at least 5, and which is convertible *in vivo* from said first to said second state whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur, for the manufacture of a diagnostic contrast medium for use in a method of diagnosis involving image generation according to a method as claimed in any one of claims 1 to 18.

22. Use as claimed in claim 21 for the manufacture of a diagnostic contrast medium for use in a method of detecting malignant melanoma, squamous cell carcinoma, sarcomas or adenocarcinomas.

PATENT COOPERATION TREATY

PTO/PCT Rec'd 20 APR 2000 PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 93.87.69118/002	FOR FURTHER ACTION	
	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB99/03488	International filing date (day/month/year) 22/10/1999	Priority date (day/month/year) 22/10/1998
International Patent Classification (IPC) or national classification and IPC A61K49/00		
Applicant NYCOMED IMAGING AS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 22/05/2000	Date of completion of this report 20.02.2001
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel. +49 89 2339 - 0 Tx: 523656 epmu d </div> </div>	Authorized officer Greif, G



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03488

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*
Description, pages:

2-9 as originally filed

1,10-22 as received on

13/01/2000 with letter of 14/12/1999

Claims, No.:

1-22 as received on

13/01/2000 with letter of 14/12/1999

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03488

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 1-10, 13-22 (all in parts), 11-12.

because:

- ☒ the said international application, or the said claims Nos. 1-10 and 13-18 (all in parts) with respect to IA, see separate sheet Item III and Item V, paragraph 5 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 1-10 and 13-22 (all in parts), 11-12.
2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/03488

- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.
2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☐ complied with.
- ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:
- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-10 and 13-22.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	13-14, 18-20, 22 (all in parts)
	No:	Claims	1-10, 15-17, 21
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10, 13-22
Industrial applicability (IA)	Yes:	Claims	19-22
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03488

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-10 and 13-18 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item IV

Lack of unity of invention

The authorized officer agrees with the objection to lack of unity (Rule 13 PCT) put forward by the International Searching Authority (see Form PCT/ISA/210 of the International Search Report).

An evaluation of novelty, inventive step and industrial applicability will only be issued the parts of claims 1-10 and 13-22 that correspond to the first (and searched) invention:

An MRI contrast agent comprising a DTPA chelate complex of a lanthanide compound having a first and a second oxydation states

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The opinion expressed as to novelty, inventive step and industrial applicability refers only to matter for which an international search report has been drawn up (Rule 66.1(e) PCT).
2. Reference is made to the following documents:
D1: WO 94 27977 A
D2: EP-A-0 450 742
D3: DE 195 07 822 A

D4: WO 96 38184 A

3. Novelty (Art. 33(2) PCT)

D1 discloses macrocyclic Europium-complexes as agents to detect temperature changes in MRI imaging in vivo, such as in the treatment of tumors (p. 1, lines 36-37; p. 2, lines 5-9 p. 4, lines 5-17; examples 1E, 2E, 3E, 4I, 5G, 6H, 11G, claims 1-6). D1 is novelty-destroying for claims 1-9 of the present application, since the properties of the complexes, such as the differences in relaxativity between the oxidation states as well as the characteristics of the states is dependent on the nature of the metal, which is the same for D1 and the present application. The subject-matter of claims 15-17 and 21 is also fully anticipated by D1.

D2 discloses Europium complexes to be used as NMR contrasting agents, to be applied in combination with a carrier such as a peptide (p. 10, lines 10-15 and 53-57; example 1C). D3 also describes Europium complexes used as magnetic resonance imaging contrast enhancers, used in the diagnosis of tumors (p. 13, lines 58-66; p. 14, lines 5-8; examples 3I, 6H, 11G). Both D2 and D3 destroy novelty of the parts of claims 1-10 relating to the first invention.

4. Inventive Step (Art. 33(3) PCT).

- 4.1. The subject-matter of claims 13 and 14 is not considered to be inventive for the following reason: D4 describes MRI contrast agents that are associated with targeting substances such as enzymes, antigens and antibodies, to be chosen on the basis of a correlation to a disease process (p. 24, line 6 to p. 25, line 18). The expert in the field would be prompted, by combining the teachings of D4 with D1, D2 or D3, to conjugate the compounds of the present application with suitable targeting substances, such as antibodies.
- 4.2. Claim 18 does not appear to be inventive since the expert in the field is familiar with the interaction between light of certain wavelengths with metal complexes and the resulting transition from one oxidation state to the other.
- 4.3. The subject-matter of claims 19 and 20 are also not inventive in the light of D4, which describes the association of a "blocking moiety" to the contrast agent, which will only be released from its association with the metal ion such as Europium once the contrast agent has reached its target site (p. 24, lines 6-11; p. 25, lines 4-5). The absence or presence of this substance acts as a trigger for converting the contrast agent from one oxidation state to the other.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03488

4.4. The subject-matter of claim 22 does not fulfill the requirements of Art. 33(3) because it is known from D1 that the MRI contrasting agents of the present application can be used for the diagnosis of tumors. The expert in the field would therefore not hesitate to apply it in the diagnosis of the diseases specified in claim 22.

5. Industrial applicability

For the assessment of the present claims 1-10 and 13-18 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 98/47539	29 October 1998	22 April 1998	22 April 1997

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

NOTIFICATION CONCERNING
DOCUMENTS TRANSMITTED

To:

The International Bureau of WIPO
34, chemin des Colombettes
CH - 1211 Geneva 20
Switzerland

Date of mailing
(day/month/year)


20.02.2001

International application No: PCT/GB99/03488

This International Preliminary Examining Authority transmits herewith the following documents:

1. ☐ demand (Rule 61.1(a)).
2. ☒ copy of the international preliminary examination report and its annexes (Rule 71.1).
3. ☐ _____ other documents (*specify*).

Name and mailing address of the IPEA/

 European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4485

Authorized officer

Senkel, H

Tel. +49 89 2399-8071



INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 11.69118/002	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 99/03488	International filing date (day/month/year) 22/10/1999	(Earliest) Priority Date (day/month/year) 22/10/1998
Applicant NYCOMED IMAGING AS et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 9 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/03488

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim(s) 1-10,13-22 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see FURTHER INFORMATION PCT/ISA/210

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10, 13-22 (PARTIALLY)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10,13-22 (partial).

An MRI contrast agent comprising a DTPA chelate complex of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

2. Claims: 1-10,
13-
22 (partial). (As far as not comprised in previous invention).

An MRI contrast agent comprising an EDTA chelate complex of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

3. Claims: 1-10,
13-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising an DTPA-BMA chelate complex of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

4. Claims: 1-10,
12-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising an D03A chelate complex of a lanthanide compound having a first and a second oxidation

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

5. Claims: 1-10,
12-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising an DOTA chelate complex of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

6. Claims: 1-10,
12-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising an HP-D03A chelate complex of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

7. Claims: 1-10,
13-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising an TMT chelate complex of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. Claims: 1-10,
13-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising an DPDP chelate complex of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

9. Claims: 1-11,
13-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising a porphyrin or porphyrin like chelate (heme, hemin) of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

10. Claims: 1-11,
13-
22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising a phtalocyanine chelate of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

11. Claims: 1-11,
13-
22 (partial). (As far as not comprised in previous inventions).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An MRI contrast agent comprising a crown ether chelate of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

12. Claims: 1-11,

13-

22 (partial). (As far as not comprised in previous inventions).

An MRI contrast agent comprising a cryptand or cryptate chelate of a lanthanide compound having a first and a second oxidation states, which differ in relaxivity by a factor of at least 5, and which is convertible in vivo from said first to said second oxidation state, said contrast agent for use in a method of imaging whereby contrast is enhanced in a body region in which conversion to said second state does or does not occur.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-10,13-22 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Moreover present claims relate to a method defined by reference to a desirable result i.e. provision of MRI contrast agents which are convertible in vivo from a first and a second oxidation state differing in relaxivity by a factor of at least 5, and capable of expressing enhanced imaging capability at a specific target site (a tumour for example).

The claims cover all compounds and methods having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT. A number of preferred compounds are mentioned that would be administered and would generate a signal in determined body regions according to the invention. No experimental data supporting this disclosure is however provided. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. An attempt is made to define the product and the method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search for the first invention has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds mentioned in the examples, with due regard to the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No

P B 99/03488

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 47539 A (GOLDING LOUISE ;NYCOMED IMAGING AS (NO); KELLAR KENNETH (US)) 29 October 1998 (1998-10-29) page 3, line 13 -page 4, line 4 page 9, line 21 page 9, line 26 claims 1,5-11 ---	1-9, 15-17, 21,22
X	WO 94 27977 A (SCHERING AG ;PLATZEK JOHANNES (DE); RADUECHEL BERND (DE); NIEDBALL) 8 December 1994 (1994-12-08) page 1, line 36 -page 3, line 6 page 4, line 5-17 claims; examples 1E,2E,3E,4I,5G,6H,11G, ---	1-9, 15-17, 21,22
X	EP 0 450 742 A (SCHERING AG) 9 October 1991 (1991-10-09) example 1C ---	1-10
-/-		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

19 April 2000

Date of mailing of the international search report

21.07.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Veronese, A

INTERNATIONAL SEARCH REPORT

International Application No

P 99/03488

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 195 07 822 A (SCHERING AG) 22 August 1996 (1996-08-22) examples 3I, 6H, 11G ---	1-10
A	DESSER T S ET AL: "DYNAMICS OF TUMOR IMAGING WITH GD-DTPA-POLYETHYLENE GLYCOL POLYMERS: DEPENDENCE ON MOLECULAR WEIGHT" JOURNAL OF MAGNETIC RESONANCE IMAGING, US, OAK BROOK, IL, vol. 4, no. 3, 1 May 1994 (1994-05-01), pages 467-472, XP000576804 cited in the application the whole document ---	1-10
A	WO 96 38184 A (CALIFORNIA INST OF TECHN ; MEADE THOMAS (US); FRASER SCOTT (US); JA) 5 December 1996 (1996-12-05) the whole document ---	1-22
A	GUNNLAUGSSON, THORFINNUR ET AL: "Luminescent europium tetraazamacrocyclic complexes with wide range pH sensitivity" CHEM. COMMUN. (CAMBRIDGE) (1998), (4), 511-512, - February 1998 (1998-02) XP002136097 the whole document -----	1-9, 15-17, 21, 22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/03488

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9847539	A	29-10-1998	AU 7066698 A EP 0977598 A	13-11-1998 09-02-2000
WO 9427977	A	08-12-1994	DE 4318369 C AT 174331 T DE 59407453 D EP 0700392 A JP 8511247 T NO 954830 A US 5747000 A	09-02-1995 15-12-1998 21-01-1999 13-03-1996 26-11-1996 28-11-1995 05-05-1998
EP 0450742	A	09-10-1991	DE 4011684 A AT 108179 T AU 644639 B AU 7418691 A CA 2039846 A DE 59102092 D DK 450742 T ES 2057746 T FI 911646 A HU 57180 A IE 65923 B JP 2854159 B JP 6065174 A NO 180582 B NZ 237711 A PT 97263 A,B US 5859214 A US 5843399 A ZA 9102550 A	10-10-1991 15-07-1994 16-12-1993 10-10-1991 07-10-1991 18-08-1994 07-11-1994 16-10-1994 07-10-1991 28-11-1991 29-11-1995 03-02-1999 08-03-1994 03-02-1997 23-12-1993 31-01-1992 12-01-1999 01-12-1998 29-01-1992
DE 19507822	A	22-08-1996	AU 4940796 A WO 9626182 A EP 0810990 A US 5676923 A	11-09-1996 29-08-1996 10-12-1997 14-10-1997
WO 9638184	A	05-12-1996	US 5707605 A AU 712899 B AU 5975196 A CA 2222974 A EP 0831928 A JP 11506455 T NO 975517 A US 5980862 A	13-01-1998 18-11-1999 18-12-1996 05-12-1996 01-04-1998 08-06-1999 19-01-1998 09-11-1999